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Research Article

Prostaglandin E2 (PGE2) and several other prostaglandins synthesized by colon suppress the proliferative activity of colonic epithelium. However, bile salts stimulate colonic epithelial proliferation despite the actions of bile salts to enhance the release of arachidonate and consequent colonic synthesis of PGE2. The current study was conducted to assess whether bile salt-induced increases in colonic formation of arachidonate metabolites other than PGE2 were linked to the stimulation of the proliferative activity of colonic epithelium. Within 10 min of addition, deoxycholate markedly stimulated the in vitro release of [¹⁴C]arachidonate from prelabeled rat colon. When given in vivo by intracolonic instillation deoxycholate (10 μmol) increased colonic accumulation of immunoreactive prostaglandin E (PGE), thromboxane B2 (TXB2), and the lipoxygenase product 12-hydroxyeicosatetraenoic acid (12-HETE) by two to fourfold over control in 30 min. This effect of intracolonic deoxycholate was followed by a ninefold increase in mucosal ornithine decarboxylase activity (4 h), and a subsequent two to threefold increase in [³H]thymidine [(³H]Thd) incorporation into DNA of either mucosal scrapings or isolated pools of proliferative colonic epithelial cells (24 h). Intracolonic instillation of indomethacin (50 μmol) suppressed to low or undetectable levels both basal colonic accumulation of PGE and TXB2 and the increases in each parameter induced by subsequent instillation of deoxycholate. By contrast, indomethacin enhanced accumulation of 12-HETE in both control colons and those [...]

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Bile Salt Stimulation of Colonic Epithelial Proliferation Evidence for Involvement of Lipoxygenase Products

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Abstract. Prostaglandin E₂ (PGE₂) and several other prostaglandins synthesized by colon suppress the proliferative activity of colonic epithelium. However, bile salts stimulate colonic epithelial proliferation despite the actions of bile salts to enhance the release of arachidonate and consequent colonic synthesis of PGE₂. The current study was conducted to assess whether bile salt-induced increases in colonic formation of arachidonate metabolites other than PGE₂ were linked to the stimulation of the proliferative activity of colonic epithelium. Within 10 min of addition, deoxycholate markedly stimulated the *in vitro* release of [¹⁴C]arachidonate from prelabeled rat colon. When given *in vivo* by intracolonic instillation, deoxycholate (10 μmol) increased colonic accumulation of immunoreactive prostaglandin E (PGE), thromboxane B₂ (TXB₂), and the lipoxygenase product 12-hydroxyeicosatetraenoic acid (12-HETE) by two to fourfold over control in 30 min. This effect of intracolonic deoxycholate was followed by a ninefold increase in mucosal ornithine decarboxylase activity (4 h), and a subsequent two to threefold increase in [³H]thymidine ([³H]Thd) incorporation into DNA of either mucosal scrapings or isolated pools of proliferative colonic epithelial cells (24 h). Intracolonic instillation of indomethacin (50 μmol) suppressed to low or undetectable levels both basal colonic accumulation of PGE and TXB₂ and the increases in each parameter induced by subsequent instillation of deoxycholate. By contrast, indomethacin enhanced accumulation of 12-HETE in both control colons and those subsequently exposed to deoxycholate. The increases in 12-HETE induced by indomethacin alone were correlated with stimulation of mucosal ornithine decarboxylase activity and [³H]Thd

incorporation into mucosal DNA. Indomethacin also enhanced the increases in these parameters induced by deoxycholate. Intracolonic instillation of phenidone (25–100 μmol) suppressed accumulation of PGE, TXB₂, and 12-HETE in control colons and the increases in these parameters induced by a subsequent instillation of deoxycholate. Phenidone alone did not alter mucosal ornithine decarboxylase activity or [³H]thymidine incorporation into mucosal DNA. However, phenidone suppressed or abolished increases in these parameters induced by a subsequent instillation of deoxycholate. 4-(2-[1H-imidazol-1-yl]ethoxy) benzoic acid hydrochloride UK 37,248, which selectively reduced colonic TXB₂ to undetectable levels without altering PGE or 12-HETE, had no effect on control or deoxycholate-induced increases in mucosal ornithine decarboxylase activity or [³H]Thd incorporation into DNA. Neither indomethacin nor phenidone altered the increases in [¹⁴C]arachidonate release induced *in vitro* by deoxycholate. Chenodeoxycholate and cholate also stimulated [¹⁴C]arachidonate release from colon *in vitro* within 10 min, and increased colonic 12-HETE (30 min) and mucosal ornithine decarboxylase activity (4 h) upon intracolonic instillation. Prior instillation of phenidone inhibited the increases in both 12-HETE and ornithine decarboxylase activity induced by these bile salts.

The results support a role for bile salt-induced increases in colonic accumulation of lipoxygenase products, as reflected by 12-HETE, in the subsequent stimulation of the proliferative activity of colonic epithelium.

Introduction

Previous studies from our laboratory have provided evidence that prostaglandins (PGs),¹ which are derived from the metab-

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1. *Abbreviations used in this paper:* df, degrees of freedom; HETE, hydroxyeicosatetraenoic acid; HPLC, high-pressure liquid chromatog-

olism of arachidonate via the cyclooxygenase pathway, act locally to suppress the proliferative activity of colonic epithelium (1). Thus, treatment of rats with the cyclooxygenase inhibitors indomethacin or aspirin stimulated DNA synthesis of colonic epithelium *in vivo* and *ex vivo*. These actions of indomethacin and aspirin were correlated with suppression of local colonic PG synthesis by the drugs (1). Moreover, prostaglandin E₂ (PGE₂), the dominant PG product of colon, as well as other PGs, inhibited colonic mucosal DNA synthesis *in vitro* (1–3). Similarly, when given orally, the stable PGE₂ analogue 16,16-dimethyl PGE₂ partially reversed the stimulatory effects of indomethacin on *in vivo* DNA synthesis in colonic mucosa (4). In view of this apparent inhibitory influence of PGE₂, it is of interest that bile salts have been shown to enhance PGE₂ release from colon (5). Yet, in direct contrast to the effects of exogenous PGE₂, bile salts stimulate colonic epithelial proliferation (6–8). Indeed, this action has been linked to the ability of bile salts to promote experimental colon carcinogenesis (9–12). Bile salts likely enhance colonic production of PGE₂ by activation of phospholipases and release of free arachidonate (13). Accordingly, in the present study, we examined the possibility that the action of bile salts to stimulate colonic epithelial proliferation might be related to an increased generation of arachidonate metabolites other than PGE₂. Specifically, the relationships between bile salt-induced increases in colonic mucosal ornithine decarboxylase (ODC) activity and DNA synthesis and an increased generation of lipoxygenase products by colon were assessed by monitoring changes in colonic accumulation of the lipoxygenase product 12-hydroxy-eicosatetraenoic acid (12-HETE). Earlier investigations in skin have implicated 12-HETE and other lipoxygenase products in both the induction of ODC and in tumor promotion in that organ (14, 15). The relationship between stimulation of colonic epithelial proliferation by bile salts and changes in colonic accumulation of thromboxane B₂ (TXB₂), which is formed as one of the end products of arachidonate metabolism via the cyclooxygenase pathway, was also examined, since thromboxanes have been reported to stimulate proliferation of some cells (16).

Our results support a link between bile salt-initiated generation of lipoxygenase products in colon and the action of bile salts to increase colonic epithelial ODC and DNA synthesis. These findings thus provide a potential explanation for the apparent paradoxical action of bile salts to stimulate both colonic PGE₂ release and epithelial cell proliferation.

Methods

Treatment of rats. Female Sprague-Dawley rats (Zivic-Miller Laboratories, Pittsburgh, PA) were fasted for the previous 8 h and throughout

raphy; KRBG, Krebs-Ringer bicarbonate buffer; ODC, ornithine decarboxylase activity; PG, prostaglandin; PGD₂, PGE, PGE₂, PGF_{1 α} , PGF_{2 α} , prostaglandins D₂, E, E₂, F_{1 α} , F_{2 α} ; [³H]Thd, [³H]thymidine; TXA₂, TXB₂, thromboxanes A₂, B₂; UK 37,248, 4-(2-[1H-imidazol-1-yl]ethoxy) benzoic acid hydrochloride.

the study period and anesthetized with pentobarbital; 50 mg/kg *i.p.* The large intestine was exposed through a midline abdominal incision. A 20-gauge needle was inserted into the lumen of the large intestine at the cecal junction. The large intestine was thoroughly flushed free of visible luminal contents with 0.9% saline that had been warmed to 37°C. A ligature was then placed externally at the ano-rectal junction to prevent escape of test solutions. Saline (2 ml), or a solution of test agents in 2 ml of saline (37°C) was instilled into the lumen of the distal colon. In some studies as indicated in the text, 30 min after insertion of the first test solution, a second instillation of 2 ml of saline or a second test agent in saline (37°C) was made. The needle puncture site was then oversewn. If animals were to be killed at 60 min after the start of the study for determination of 12-HETE, prostaglandin E (PGE), and TXB₂, they were maintained under pentobarbital anesthesia for the entire 60 min. For subsequent determination of ODC activity (at 4 h) or [³H]thymidine ([³H]Thd) incorporation into DNA (at 24 h), the abdominal incision was closed with wound clips. At timed intervals after treatment, as indicated in Results, control and experimental rats were again anesthetized with pentobarbital, 50 mg/kg *i.p.* The distal colons were resected from the colonic flexure to 1 cm above the anal orifice, and further studies were conducted as described below. A portion of each distal colon was fixed in 10% buffered formalin and sections were examined by light microscopy after staining with hematoxylin and eosin. This method of administration of bile salts was used because in our hands it gave much more consistent increases in ODC and DNA synthesis than the previously reported intrarectal method of administration of bile salts (8).

Determination of colonic release of PGE, TXB₂, 12-HETE, and malondialdehyde ex vivo. In these studies, rats were killed 30 min after intracolonic instillation of bile salts or saline and the distal colon was resected and placed in saline at 4°C. Three segments (100–150 mg) from each distal colon were incubated in a 25-ml flask in a Dubnoff metabolic shaker at 37°C for 30 min. Each segment was placed in a flask containing 2 ml of Krebs-Ringer bicarbonate buffer with 1 mg/ml glucose (KRBG) and 95% O₂/5% CO₂ as the gas phase (17). At the end of 30 min, an aliquot of each incubation medium was immediately assayed for malondialdehyde. Residual media were frozen at –20°C for subsequent assay of PGE, TXB₂, and 12-HETE.

Determination of colonic content of PGE, TXB₂, and 12-HETE in vivo. 30 min after intracolonic instillation of bile salts or saline, the distal colons were excised and quick-frozen within 2 s between clamps cooled with liquid nitrogen. The frozen tissue was weighed and powdered in a percussion mortar and cooled with liquid nitrogen, without allowing the tissue to thaw. The frozen powder was then extracted with 10 vol of ethyl acetate, which contained 100 µg/ml of butylated hydroxytoluene and 3.5% formic acid. [³H]12-HETE and [³H]PGE₂ were added to assess recovery. The ethyl acetate extract was evaporated to dryness and resuspended in 0.5 ml of methanol/water/acetic acid, 78:22:0.01, vol/vol. A 200-µl aliquot of the sample was then applied to a Waters µ Bondapak reverse phase C-18 column (Waters Associates, Inc., Milford, MA) and elution-conducted at a flow rate of 1 ml/min with methanol/water/acetic acid, 78:22:0.01, as previously described in detail (18). Under the conditions used, [³H]12-HETE was eluted in a sharp peak at 9–10 min and was well separated from both [³H]PGE₂ (the predominant prostaglandin product in whole colon), which had a retention time of 5 min and from arachidonic acid, with a retention time of 15 min. [³H]12-HETE was not separated from labeled 5- or 15-HETE under the conditions used. The appropriate peak fractions from the high-pressure liquid chromatography (HPLC) column were evaporated to dryness under N₂ and resuspended in 10 mM phosphate-buffered saline, which contained 1% rabbit serum for

radioimmunoassay of 12-HETE and PGE. Under the conditions used, recovery of [³H]12-HETE or [³H]PGE was routinely 45–55%.

Radioimmunoassay of PGE, TXB₂, and 12-HETE. PGE and TXB₂, the stable degradation product of thromboxane A₂ (TXA₂), were determined as previously described (1, 19), using antisera obtained from Regis Chemical Co., Morton Grove, IL and New England Nuclear, Boston, MA, respectively. 12-HETE was determined using antisera obtained from Seragen, Inc., Boston, MA. Standard curves were plotted as logit-log functions from 0.1–10 ng PGE₂, 5–250 pg TXB₂, and 10–2000 pg 12-HETE. The cross-reactivity of PGE₂, prostaglandin D₂ (PGD₂), prostaglandin F_{2α} (PGF_{2α}), prostaglandin F_{1α}, 6-keto PGF_{1α}, arachidonate, and 12-HETE with the TXB₂ antisera was ≤0.2%. Cross-reactivity of PGD₂, PGF_{2α}, and 6-keto PGF_{1α} in the PGE assay was 1–2% and that of TXB₂, arachidonate, and 12-HETE was ≤0.5%. Cross-reactivity of PGE₂, PGD₂, PGF_{2α}, 6-keto PGF_{2α}, TXB₂ and arachidonate in the 12-HETE assay was no more than 0.1%. Under the conditions used, the radioimmunoassay values obtained varied linearly over a 10-fold range of dilutions.

Determination of malondialdehyde. Malondialdehyde content of the medium was determined by the thiobarbituric acid method (20). A 1-ml aliquot of medium was extracted at 4°C with an equal volume of 10% trichloroacetic acid in 0.3 N HCl. The extracts were then centrifuged at 1,500 g for 15 min. A 1-ml aliquot of the clear supernatant was added to 200 μl of 0.12 M thiobarbituric acid in 0.26 M Tris. Ferrous sulfate and butylated hydroxytoluene was also added to the reaction mixtures at final concentrations of 2.5 and 0.25 mM, respectively (20). The mixtures were heated at 95°C for 15 min and the absorbance of each sample determined at 532 nm. Standard curves with malondialdehyde bis (dimethylacetal) were linear between 0.25 and 2.5 nmol in 1.2 ml of assay mixture.

Determination of ODC activity. Unless otherwise stated, rats were routinely killed 4 h after intraluminal instillation of bile salts or saline. The mucosal surface of the distal colon was scraped and 150 mg wet weight was homogenized in 1 ml of 50 mM sodium phosphate, pH 7.4, which contained 0.1 mM pyridoxal phosphate and 0.1 mM EDTA. Homogenates were centrifuged at 38,000 g for 20 min and the supernatants were assayed immediately. Enzyme activity was determined from the release of ¹⁴CO₂ from DL[1-¹⁴C]ornithine, as previously described (21). Briefly, reaction mixtures contained 0.02 mM pyridoxal phosphate, 4 mM dithiothreitol, 1 mM EDTA, 0.4 mM L-ornithine, 40 mM sodium phosphate (pH 7.2), 100 μl of the colonic mucosal soluble fraction (~100 μg of protein) and 0.5 μCi of DL[1-¹⁴C]ornithine in a final volume of 0.25 ml. Assays were done in duplicate. Blanks contained buffer alone or the heated (95°C for 10 min) soluble fraction. Results were corrected for the blank value obtained. ODC was linear with time for 60 min with 20–200 μg of protein under all conditions of study.

Determination of [³H]Thd incorporation into DNA in vivo. Rats were treated with test agents intracolonic as described above. [³H]Thd (100 μCi/kg) was injected intraperitoneally 22 h after the deoxycholate or saline instillation had been made. Rats were killed 2 h after the injection of [³H]Thd. Distal colons were excised and placed in saline at 4°C. The mucosa was scraped and DNA content was extracted, counted, and assayed as previously described (1, 22).

Determination of ex vivo incorporation of [³H]Thd into DNA. Rats were treated intracolonic with test agents as described above. Unless otherwise indicated, rats were killed 24 h after instillation of deoxycholate or saline and segments (1 × 1 cm) prepared from the distal colons. Three segments from each rat colon were incubated in 25-ml flasks in a Dubnoff metabolic shaker for 60 min at 37°C. Each flask contained 2 ml of KRBG and [³H]Thd (0.5 μCi/ml), with 95% O₂/5% CO₂ as

the gas phase. At the end of the incubation, the mucosal surface was scraped and DNA content was extracted, assayed, and counted as previously described (1, 22). Protein was determined by the method of Lowry (23).

Assessment of the effects of bile salts on colonic release of [¹⁴C]arachidonate in vitro. Segments of rat distal colon were labeled by incubation with 0.5 μCi (4.5 μM) of [¹⁴C]arachidonate in KRBG at 37°C in a metabolic shaker as outlined above. Segments were then transferred to fresh KRBG without label for 15 min. Fatty acid-free albumin was present during the postlabeling incubation period to remove free labeled arachidonate. Segments were then transferred to fresh KRBG plus fatty acid-free albumin, and aliquots of the media were removed for counting at 10-min intervals to determine the basal rate of release of [¹⁴C]arachidonate from membrane lipids. Deoxycholate (10 μmol) or other bile salts were added to the 2-ml incubation media (final concentration 5 mM), and aliquots of the media were removed for counting at 10-min intervals.

Isolation of superficial and proliferative pools of rat distal colonic epithelium and autoradiographic analysis of the sites of DNA synthesis by rat distal colonic epithelium were performed as previously reported in detail (1, 24).

Materials. Reagents and antisera for radioimmunoassay of 12-HETE were obtained from Seragen, Inc. DL[1-¹⁴C]Ornithine (30–55 mCi/mmmole), [³H]5-, 12-, and 15-HETE (30–100 Ci/mmmole) were obtained from New England Nuclear. Phenidone, deoxycholate, cholate, and chenodeoxycholate were purchased from Sigma Chemical Co., St. Louis, MO. Benoxaprofen and 4-(2-[IH-imidazol-1-yl]ethoxy) benzoic acid hydrochloride (UK 37,248) were the generous gifts of Eli Lilly Co., Indianapolis, IN and Upjohn Co., Kalamazoo, MI, respectively.

Statistics. Statistical significance of differences between mean values was determined by the *t* test for unpaired data. Studies were conducted on four to eight rats in each experimental group as indicated in the text. Between 8 and 12 rats could be studied on any given day. Experiments were thus repeated until the indicated number of rats in each group had been studied. For the purposes of statistical analysis, the average of replicate determinations for any parameter from a single rat colon was entered as a single value.

Results

Fig. 1 compares the effects in vitro of 5 mM deoxycholate (10 μmol in 2 ml media), chenodeoxycholate, and cholate on the release of [¹⁴C]arachidonate from incubated distal colonic segments prelabeled with [¹⁴C]arachidonate. Deoxycholate markedly increased ¹⁴C release from colon prelabeled with [¹⁴C]arachidonate. Increases in [¹⁴C]arachidonate release occurred within 10 min of addition of deoxycholate or the other bile salts tested. At 5 mM, deoxycholate was a more potent stimulus of [¹⁴C]arachidonate release than an equimolar concentration of either chenodeoxycholate or cholate (Fig. 1). The greater potency of deoxycholate as a stimulus of [¹⁴C]arachidonate release from colon was also evident when this action of the three bile salts was examined in vitro at concentrations of 2, 10, or 15 mM (not shown). Accordingly, most of the in vivo studies described below were conducted with deoxycholate.

Table I illustrates the effects of intracolonic instillation of saline or deoxycholate on ODC in 38,000-g soluble fractions

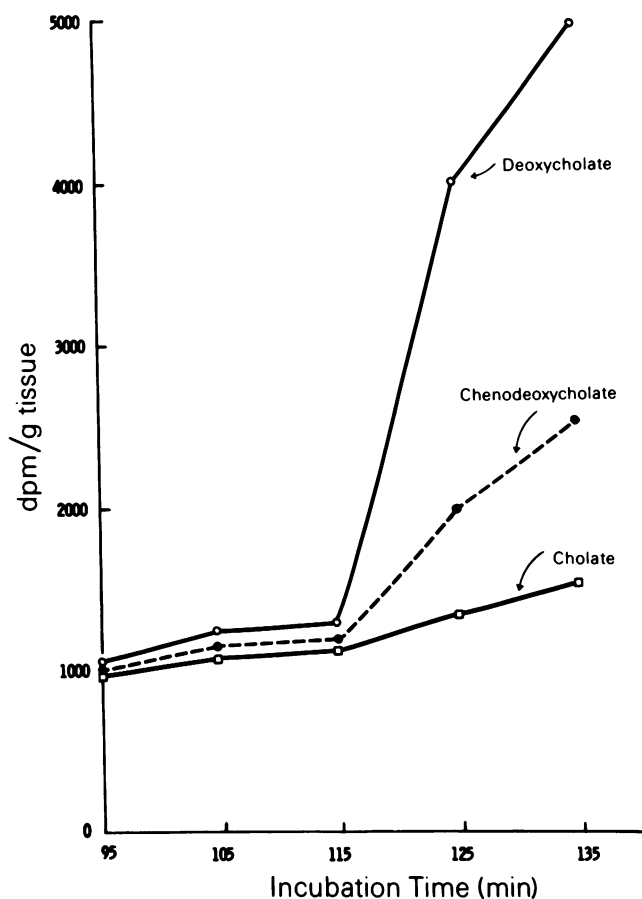


Figure 1. Colonic segments (100–150 mg) were preincubated for 30 min in 2 ml of KRBG, 95% O₂/5% CO₂ with [¹⁴C]arachidonate. Segments were transferred to fresh KRBG at *t* = 30, 50, and 75 min. Media (50 μl) was withdrawn and counted at *t* = 95, 105, and 115 min. At *t* = 115, sodium deoxycholate, chenodeoxycholate or chololate was added to a final concentration of 5 mM. A 50-μl aliquot of the media was withdrawn at *t* = 125 and 135 min, and counted. Results shown are means of triplicate determinations from a single experiment verified in three separate studies.

of colonic mucosal homogenates. Alterations in [³H]Thd incorporation into mucosal DNA, examined in vivo and ex vivo, are also shown. Four groups of rats each were studied 4 or 24 h after operative intracolonic instillations. A fifth group of rats was not surgically treated. As shown, ODC of colon mucosa and [³H]Thd incorporation into mucosal DNA of rats receiving operative intracolonic instillation of saline did not differ from corresponding values of unoperated control rats at 4 or 24 h after saline instillation. Instillation of 10 μmol of deoxycholate in 2 ml of saline increased ODC of colonic mucosa ninefold at 4 h over that of the saline-treated or unoperated controls. ODC had returned to control levels by 24 h after deoxycholate treatment. In separate studies, ODC was also examined 30 min after intracolonic instillation of deoxycholate. At this earlier time point, no increase in ODC

Table I. Effects of Intracolonic Deoxycholate on ODC Activity and [³H]Thd Incorporation into DNA In Vivo and Ex Vivo

Treatment	ODC <i>pmol CO₂/min/mg protein</i>	[³ H]Thd incorporation into DNA	
		In vivo	Ex vivo
No surgery	2.2±0.4	10±1	17±3
Saline, 4 h	3.1±0.5	13±2	21±3
Deoxycholate, 4 h	26±4*	12±2	19±2
Saline, 24 h	2.8±0.4	14±2	23±4
Deoxycholate, 24 h	3.2±0.6	31±5*	38±6*

Saline (2 ml) or 10 μmol of deoxycholate dissolved in 2 ml of saline was administered intracolonic to four groups of rats as described in Methods. One group of rats was not surgically treated. The same rats were used for studies of ODC activity and ex vivo [³H]Thd incorporation into DNA, while separate groups of rats were used for studies of in vivo [³H]Thd. Rats were killed after 4 or 24 h and ODC activity was determined in the 38,000-g soluble fraction of distal colonic mucosal scraping homogenates. [³H]Thd incorporation into DNA was determined in vivo and ex vivo 4 or 24 h after intracolonic administration of saline or deoxycholate as indicated. For determination of in vivo [³H]Thd incorporation into DNA, rats were injected with 100 μCi/kg i.p. of [³H]Thd 2 h before death. For determination of ex vivo [³H]Thd incorporation into DNA, three segments (100–150 mg) from each distal colon were incubated for 60 min in 2 ml each of KRBG, 95% O₂ 5% CO₂ with 0.5 μCi/ml of [³H]Thd. At the end of the incubation, the mucosal surface was scraped and DNA content was extracted, assayed, and counted as described in Methods. Results shown are means±SE of determinations from eight rats in each study group (degrees of freedom [df] = 14 comparing any 2 parameters by the independent *t* test).

* *P* < 0.01 compared with corresponding value in rats treated with saline.

was observed in response to deoxycholate (data not shown). As is also shown in Table I, intracolonic administration of deoxycholate did not alter in vivo [³H]Thd incorporation into DNA when examined at 4 h, but increased incorporation twofold by 24 h compared with either the saline or unoperated controls. Since intracolonic administration of saline or deoxycholate might influence delivery of systemically administered [³H]Thd to the colon, the effect of intracolonic saline and deoxycholate treatment on [³H]Thd incorporation into mucosal DNA was examined ex vivo. In these studies rats were treated intracolonic with test agents as indicated. The distal colons were then excised and segments were incubated for 60 min with [³H]Thd ex vivo. Analogous to the results obtained when [³H]Thd was administered in vivo, deoxycholate increased ex vivo [³H]Thd incorporation into DNA approximately twofold at 24 h, compared with values in saline or unoperated controls (Table I).

Fig. 2 illustrates the dose-response relationship between intracolonic deoxycholate and ODC. A significant (threefold)

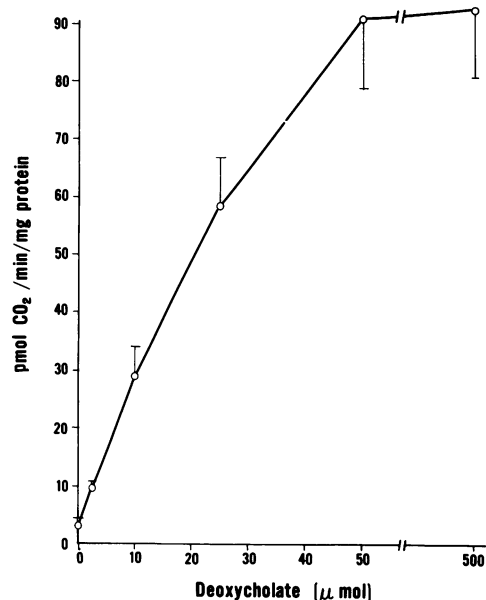


Figure 2. Dose-response relationship between intracolonic deoxycholate and ornithine decarboxylase activity in colonic mucosal 38,000-g soluble fractions. Saline (2 ml) or the indicated dose of deoxycholate dissolved in 2 ml of saline was instilled intracolonic to five groups each containing four rats, as described in Methods. After 4 h, ODC activity was determined in the 38,000-g soluble fraction of scrapings of distal colonic mucosa. Results shown are means \pm SE of duplicate determinations on soluble fractions prepared from four rat colons in each group.

increase in enzyme activity was observed with 2.5 μ mol of deoxycholate. Increasing deoxycholate from 2.5 to 50 μ mol resulted in a progressive increase in ODC. No further rise in ODC occurred when deoxycholate was increased to 100 μ mol. When tested over the same dose range (5–100 μ mol) chenodeoxycholate and cholate were less potent than deoxycholate in increasing ODC in colonic mucosa (deoxycholate > chenodeoxycholate > cholate, data not shown).

Histologic examination by light microscopy showed no morphologic changes in distal colon prepared from rats 4 or 24 h after instillation of saline or 4 h after instillation of 10 μ mol of deoxycholate. Specifically, no inflammatory reaction was observed at 4 h. By 24 h after instillation of 10 μ mol of deoxycholate, there was some depletion of goblet cells. There was also mild infiltration of the submucosa and lamina propria with neutrophils, lymphocytes, and a few histiocytes and plasma cells. The epithelial surface remained largely intact, with rare areas of focal loss of surface epithelium by 24 h. Raising the dose of deoxycholate to 50 μ mol caused a general depletion in goblet cells, more marked inflammation, edema, and focal loss of surface epithelium by 4 h. Accordingly, all subsequent studies were conducted with 10 μ mol of deoxycholate, which produced a consistent 8- to 10-fold increase in ornithine decarboxylase activity at 4 h, without any evidence of histologic change by light microscopy at this time.

To examine the effect of intracolonic instillation of deoxycholate on several intermediates or products of colonic arachidonate metabolism, groups of four rats each were first treated with intracolonic instillation of 2 ml of saline, or with 50 μ mol of indomethacin, phenidone, or UK 37,248, each dissolved in 2 ml of saline. Indomethacin inhibits cyclooxygenase activity (25), whereas phenidone has been shown to inhibit both cyclooxygenase and lipoxygenase activities (26). UK 37,248 is an inhibitor of thromboxane synthetase activity (27). After 30 min, a second instillation of either 10 μ mol of deoxycholate in 2 ml of saline or of 2 ml of saline alone was made. Since prior studies in colon and other tissues have demonstrated that PGs are not stored but are rapidly released from cells upon synthesis (10, 28), release of PGE from colon was examined *ex vivo* as previously reported (1), and as described above. As shown in Table II, media PGE, TXB₂, and malondialdehyde were higher in incubates of segments from deoxycholate-treated rats compared with that of rats that had been treated with saline. PGE accumulation in the media was reduced by 70–80% in incubates of segments prepared from rats treated with indomethacin or phenidone, with or without an *in vivo* exposure to deoxycholate. By contrast, intracolonic instillation of UK 37,248 did not influence PGE accumulation in the media under any of the conditions

Table II. Effects of Intracolonic Instillation of Inhibitors of Arachidonate Metabolism on Basal and Deoxycholate-induced Changes in PGE, TXB₂, and Malondialdehyde Accumulation in Media of Colonic Segments Incubated *Ex Vivo*

Intracolonic instillation		PGE	TXB ₂	MDA
No. 1 (<i>t</i> = 0)	No. 2 (<i>t</i> = 30)			
<i>ng/mg tissue</i>				
Saline	Saline	0.32 \pm 0.04	0.058 \pm 0.012	0.16 \pm 0.02
Indomethacin	Saline	0.10 \pm 0.01*	<0.001	0.062 \pm 0.009*
Saline	Deoxycholate	1.2 \pm 0.2*	0.091 \pm 0.015*	0.48 \pm 0.08*
Indomethacin	Deoxycholate	0.13 \pm 0.02*‡	<0.001	0.26 \pm 0.05*‡
Phenidone	Saline	0.13 \pm 0.02*	<0.001	0.017 \pm 0.002*
Phenidone	Deoxycholate	0.17 \pm 0.03*‡	<0.001	0.039 \pm 0.005*‡
UK 37,248	Saline	0.34 \pm 0.05	<0.001	0.14 \pm 0.02
UK 37,248	Deoxycholate	1.3 \pm 0.02*	<0.001	0.52 \pm 0.07*

At *t* = 0, 2 ml of saline or indomethacin (50 μ mol), phenidone (50 μ mol), or UK 37,248 (50 μ mol) dissolved in 2 ml of saline was instilled intracolonic in rats as indicated. Controls received saline alone. At *t* = 30 min, 2 ml of saline alone or 10 μ mol of deoxycholate in 2 ml saline was instilled. 30 min after the second instillation, the rats were killed, distal colons were removed and rinsed in chilled saline. Three segments (100–150 mg) from each rat were prepared and incubated *ex vivo* for 30 min in 2 ml of KRBG, 95% O₂, 5% CO₂. Media PGE, TXB₂, and malondialdehyde (MDA) accumulation were determined by radioimmunoassay as described in Methods. Results shown are means \pm SE of single determinations on three incubates from each of six rats (df = 10 for comparing any two parameters by the independent *t* test).

* *P* < 0.01 compared with corresponding value after instillation of saline alone.

‡ *P* < 0.01 compared with corresponding value for deoxycholate after initial instillation of saline alone.

examined in Table II. Intracolonic administration of indomethacin, phenidone, or UK 37,248 reduced TXB₂ accumulation in the media to undetectable levels, in both colon incubates from saline controls and from rats treated with deoxycholate. As is also shown in Table II, indomethacin suppressed malondialdehyde accumulation 45–60%, whereas phenidone suppressed malondialdehyde by 90% in colon incubates from control or deoxycholate-treated rats. By contrast, treatment of rats with UK 37,248 was without effect on colonic malondialdehyde formation *ex vivo* (Table II). Immunoreactive 12-HETE did not differ in the incubation media under any of the experimental conditions studied in Table II (not shown).

Previous studies in other tissues have found that HETEs, products of lipoxygenase activity, are not readily released extracellularly in a fashion analogous to PGs, but are largely retained in cells or bound to cell membranes (29). Accordingly, immunoreactive 12-HETE accumulation was assessed *in vivo* in extracts of control and experimental distal colons that had been quick frozen in liquid N₂. Correlative measurements of PGE and TXB₂ were made in the same tissue extracts. The results are presented in Table III. Rats were first treated with saline, indomethacin, phenidone, or UK 37,248, with a subsequent instillation of saline or deoxycholate, as described in

Table III. Effects of Intracolonic Instillation of Inhibitors of Arachidonate Metabolism on Basal and Deoxycholate-induced Changes in PGE₂, TXB₂, and 12-HETE Content of Quick-Frozen Colon

Intracolonic instillation				
No. 1 (t = 0)	No. 2 (t = 30 min)	PGE	TXB ₂	12-HETE
			ng/g tissue	
Saline	Saline	28±5	5±1	2.2±0.5
Indomethacin	Saline	5±1*	<1	3.7±0.6*
Saline	Deoxycholate	64±8*	9±2*	6.2±0.8*
Indomethacin	Deoxycholate	8±2*	<1	9.3±1*‡
Phenidone	Saline	9±2*	<1	0.8±0.1*
Phenidone	Deoxycholate	12±2*	<1	0.9±0.2*‡
UK 37,248	Saline	33±6	<1	2.5±0.6
UK 37,248	Deoxycholate	59±10*	<1	5.7±0.9*

Rats were treated as described in Table II legend. At t = 60 min, the distal colons were excised and immediately frozen between liquid N₂ cooled clamps. The frozen tissue was weighed, powdered in a liquid N₂ cooled percussion mortar, and extracted with 10 vol of ethyl acetate, which contained 100 µg/ml butylated hydroxytoluene, 3.5% formic acid. [³H]12-HETE and [³H]PGE₂ were added to assess recovery. The extract was evaporated and resuspended in the mobile phase for HPLC as described in Methods. The HPLC fractions containing [³H]12-HETE and [³H]PGE₂ were collected, evaporated to dryness and resuspended in phosphate-buffer saline, 1% rabbit serum for radioimmunoassay. Results shown are means±SE of determinations on extracts from four distal colons in each group (df = 6 comparing any two parameters by independent t test).

* P < 0.01 compared with corresponding value after instillation of saline alone.
‡ P < 0.01 compared with corresponding value for deoxycholate after initial instillation of saline alone.

the footnotes to Table II. 30 min after instillation of deoxycholate or saline the colons were quick-frozen between liquid N₂ cooled clamps within 2 s of excision. As shown in Table III, deoxycholate increased colonic PGE, TXB₂, and 12-HETE accumulation two to fourfold. Indomethacin suppressed accumulation of PGE by 70–80% in both saline and deoxycholate-treated colons, and reduced colon TXB₂ to undetectable levels in both groups. By contrast, indomethacin increased 12-HETE accumulation by 60–70% in both saline and deoxycholate-treated colons. Prior intracolonic instillation of phenidone suppressed PGE, TXB₂, and 12-HETE accumulation by 70–100%, in both control colons and those with a subsequent exposure to deoxycholate. As is also shown in Table III, UK 37,248 was without effect on either *in vivo* colonic PGE or 12-HETE accumulation, but reduced TXB₂ to undetectable levels.

Table IV illustrates the effects of intracolonic instillation of indomethacin, phenidone, or UK 37,248 on colonic mucosal ODC and on *in vivo* [³H]Thd incorporation into DNA. Rats were treated as described in Table II. ODC of mucosa was determined in colons from rats killed 4 h after the second instillation (saline or deoxycholate). For *in vivo* measurement of [³H]Thd incorporation into DNA, rats were killed 24 h after the second instillation. [³H]Thd (100 µCi/kg) was injected 2 h before death. As shown in Table IV, intracolonic administration of deoxycholate increased mucosal ODC approximately

Table IV. Effects of Intracolonic Administration of Inhibitors of Arachidonate Metabolism in the Presence and Absence of Deoxycholate on Ornithine Decarboxylase Activity and In Vivo [³H]Thd Incorporation into DNA

Intracolonic instillation				
No. 1 (t = 0)	No. 2 (t = 30 min)	ODC	[³ H]Thd	
		pmol CO ₂ / min/mg protein	dpm/µg DNA	
Saline	Saline	3.5±0.6	13±2	
Indomethacin	Saline	11±2*	24±3*	
Saline	Deoxycholate	32±5*	27±4*	
Indomethacin	Deoxycholate	56±7*‡	49±7*‡	
Phenidone	Saline	3.4±0.5	11±2	
Phenidone	Deoxycholate	3.6±0.7‡	13±2‡	
UK 37,248	Saline	3.2±0.4	12±1	
UK 37,248	Deoxycholate	39±7*	24±4*	

Rats were treated with the agents shown above, as described in Table II legend. For determination of ODC activity, rats were killed 4 h after the second instillation and enzyme activity was determined in 38,000-g soluble fractions of colonic mucosal scraping homogenates as described in Methods. For determination of *in vivo* [³H]Thd incorporation into DNA, rats were injected with 100 µCi/kg i.p. [³H]Thd 2 h before death. Rats were killed 24 h after the second instillation, and mucosal DNA was extracted, assayed, and counted. Results shown are means±SE of determinations on extracts from six rats in each group (df = 10 comparing any two parameters by the independent t test).

* P < 0.01 compared with corresponding value after instillation of saline alone.
‡ P < 0.01 compared with corresponding value for deoxycholate after initial instillation of saline alone.

10-fold, and increased in vivo [^3H]Thd incorporation into DNA twofold over values in rats exposed to saline alone. Intracolonic indomethacin significantly enhanced both ODC and [^3H]Thd incorporation into mucosal DNA compared with corresponding values in saline-treated controls. Moreover, prior intracolonic indomethacin also enhanced stimulation of both parameters induced by a subsequent instillation of deoxycholate. Phenidone alone had no effect on basal ODC at 4 h or [^3H]Thd incorporation into DNA at 24 h. However, prior instillation of phenidone abolished the stimulatory effect of a subsequent instillation of 10 μmol deoxycholate on both of these parameters. As is also shown in Table IV, UK 37,248 had no influence on colonic mucosal ODC or on in vivo [^3H]Thd incorporation into mucosal DNA in rats that subsequently received either saline or deoxycholate. In separate experiments (not shown) intracolonic administration of 50 or 100 μmol of benoxaprofen, a relatively specific inhibitor of 5-HETE production in polymorphonuclear cells (30), did not reduce basal 12-HETE content of quick-frozen colon and did not alter basal ODC or in vivo [^3H]Thd incorporation into DNA. Moreover, prior intracolonic instillation of 50 or 100 μmol benoxaprofen failed to alter the increases in colonic 12-HETE, mucosal ODC, or mucosal [^3H]Thd incorporation into DNA induced by a subsequent instillation of 10 μmol of deoxycholate.

Instillation of 50 μmol of indomethacin or phenidone alone produced no histologic alteration in distal colon compared with colons from saline controls, as assessed by light microscopic examination of colons removed at 1, 4, or 24 h after administration of these agents. Moreover, prior treatment with either indomethacin or phenidone did not detectably alter the histologic changes observed 24 h after instillation of 10 μmol of deoxycholate (goblet cell depletion and mild inflammation).

Table V shows alterations in in vivo [^3H]Thd incorporation into DNA induced in superficial vs. proliferative cells isolated from control and experimental rat colons. Intracolonic instillations were performed as described in Table II legend, with [^3H]Thd injected 2 h before death as described in Table IV. Analogous to the findings in colonic mucosal scrapings, instillation of either indomethacin or deoxycholate alone significantly increased (two to threefold) [^3H]Thd incorporation into DNA extracted from the isolated proliferative cell pool at 24 h. Prior instillation of indomethacin enhanced the increases in [^3H]Thd incorporation induced in proliferative cells by deoxycholate. Phenidone alone did not alter [^3H]Thd incorporation into DNA of isolated colonic epithelial cells. However, prior instillation of phenidone abolished increases in this parameter induced in the proliferative cell pool by deoxycholate. At 24 h, none of the test agents had caused significant alterations in incorporation of [^3H]Thd into DNA by isolated superficial colonic epithelial cells. Consistent with the foregoing observations in isolated epithelial cells, autoradiographic studies of distal colons from rats treated with indomethacin, deoxycholate or both indicated that enhanced DNA synthesis occurred predominantly in epithelial cells of the lower third of the crypts (not shown).

Table V. Effects of Indomethacin and Phenidone on Deoxycholate-induced Changes in In Vivo [^3H]Thd Incorporation into DNA of Isolated Superficial and Proliferative Colonic Epithelial Cells

Intracolonic instillation			
No. 1 ($t = 0$)	No. 2 ($t = 30$ min)	Superficial cells	Proliferative cells
		dpm/ μg DNA	dpm/ μg DNA
Saline	Saline	3.1 \pm 0.6	25 \pm 5
Indomethacin	Saline	4.9 \pm 0.8	62 \pm 9*
Saline	Deoxycholate	4.6 \pm 0.8	71 \pm 8*
Indomethacin	Deoxycholate	5.0 \pm 0.7	98 \pm 10*‡
Phenidone	Saline	2.5 \pm 0.6	21 \pm 5
Phenidone	Deoxycholate	3.4 \pm 0.6	29 \pm 6‡

Rats were treated with the agents indicated above as described in Table II legend. For determination of [^3H]Thd incorporation into DNA, rats were injected with 100 $\mu\text{Ci}/\text{kg}$ i.p. [^3H]Thd 2 h before death. Rats were killed 24 h after the second instillation. Superficial and proliferative cell pools of colonic epithelium were then isolated as described in Methods. Each experimental group consisted of eight rats. Epithelial cells isolated from colons of two rats of the same experimental group were pooled before extraction and assay of DNA and entered as a single value for purposes of statistical analysis. Results are means \pm SE (df = 6). * $P < 0.01$ compared with corresponding value after instillation of saline alone. ‡ $P < 0.01$ compared with corresponding value for deoxycholate after initial instillation of saline alone.

The possibility that phenidone interfered with delivery to the colon of systemically administered [^3H]Thd was considered. Accordingly, the influence of intracolonic administration of phenidone with or without deoxycholate on ex vivo [^3H]Thd incorporation into DNA was examined. Rats were treated with phenidone or deoxycholate alone or with phenidone followed by deoxycholate as described in the footnote to Table II. Colons were excised 24 h later and segments incubated with [^3H]Thd ex vivo, as described in Table I legend. Analogous to results obtained in vivo, intracolonic phenidone alone did not influence ex vivo incorporation of [^3H]Thd into mucosal DNA in segments of colon incubated ex vivo compared with that observed in segments from saline-treated rats. However, prior treatment with phenidone abolished increases in ex vivo [^3H]Thd incorporation induced by subsequent intracolonic deoxycholate (saline, 17 \pm 2; deoxycholate, 29 \pm 4; phenidone, 18 \pm 3; phenidone followed by deoxycholate, 16 \pm 2 dpm/ μg DNA). In other in vitro studies, conducted as described in Fig. 1 legend, phenidone (50 $\mu\text{mol}/2$ ml incubation media) did not influence the release of [^{14}C]arachidonate induced by deoxycholate from prelabeled colonic segments when added directly to the incubates 30 min before in vitro addition of deoxycholate (– phenidone, – deoxycholate, 22 \pm 3; – phenidone, + deoxycholate, 586 \pm 74; + phenidone, – deoxycholate, 27 \pm 4; + phenidone, + deoxycholate, 523 \pm 59 dpm/min per g tissue). Moreover, when added directly to the assay mixture, phenidone (50 $\mu\text{mol}/2$ ml) had no effect on ODC in soluble fractions of mucosal homogenates obtained from saline or deoxycholate-treated rats (not shown).

To examine the relationship between phenidone suppression of deoxycholate-stimulated colonic 12-HETE accumulation and mucosal ODC, the dose-response relationship between phenidone and 12-HETE accumulation or ODC activity was studied in saline and deoxycholate-treated rats. Saline, or the indicated amount of phenidone dissolved in saline, was initially instilled intracolonic. After 30 min, a second instillation of saline or deoxycholate was made. The 12-HETE content of quick-frozen colon and ODC in soluble fractions of mucosal homogenates were determined 30 min and 4 h, respectively, after the second instillation. As shown in Fig. 3, phenidone alone did not alter basal ODC (no exposure to deoxycholate) at any of the doses tested. However, phenidone at 25 μmol or greater did suppress basal 12-HETE accumulation. Prior instillation of 25 μmol phenidone suppressed both the increases in 12-HETE and ODC induced by deoxycholate. Moreover,

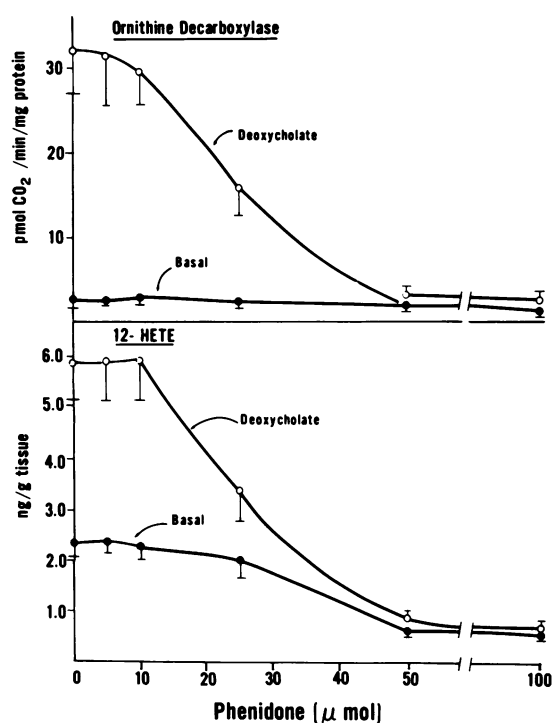


Figure 3. Dose-response relationship between intracolonic phenidone and ornithine decarboxylase activity of 12-HETE in the presence and absence of deoxycholate. Saline (2 ml) or the indicated dose of phenidone dissolved in 2 ml of saline was first administered intracolonic. After 30 min, 2 ml of saline alone (basal) or 10 μmol of deoxycholate in 2 ml of saline was administered. For determination of 12-HETE, rats were killed 30 min after the second instillation, the distal colons were quick-frozen between clamps cooled with liquid N_2 and 12-HETE was extracted, purified by HPLC, and assayed, as described in the footnote to Table III. For determination of ornithine decarboxylase, rats were killed 4 h after the second instillation and enzyme activity was determined on the 38,000-g soluble fraction of scrapings of colonic mucosa. Results shown are means \pm SE of duplicate determinations on supernatant fractions of four distal colons.

doses of 50 or 100 μmol of phenidone abolished the stimulatory effects of deoxycholate on both these parameters.

Table VI shows the effects of phenidone on increases in 12-HETE and ODC induced by chenodeoxycholate or cholate. As noted above these two bile salts were less potent than deoxycholate in stimulating ODC. Indeed, 10 μmol of cholate did not significantly increase ODC or 12-HETE (not shown). Thus, the response of these parameters to 20 μmol of cholate are given in Table VI. Chenodeoxycholate (10 μmol) or cholate (20 μmol) increased both 12-HETE and ODC, although responses were less than that observed with 10 μmol of deoxycholate (Fig. 3). Phenidone abolished increases in 12-HETE and ODC induced by either 10 μmol of chenodeoxycholate or 20 μmol of cholate (Table VI).

Discussion

Consistent with earlier studies (8, 31), exposure of colonic mucosa to deoxycholate and other bile salts in vivo results in marked and rapid increases in mucosal ODC, and a subsequent stimulation of mucosal DNA synthesis (Table I). The increase in ODC, the rate-limiting enzyme for the biosynthesis of polyamines (32), is likely an essential prerequisite step to the enhancement of proliferative activity (32–35). The bile salt-induced increase in colonic mucosal DNA synthesis is confined to the normal proliferative zone of the crypt (lower third), as demonstrated by autoradiography and by [^3H]Thd incorporation into DNA in isolated proliferative and superficial colonic epithelial cells (Table V). Acute effects of other test agents on

Table VI. Effects of Phenidone on Increases in 12-HETE and ODC Activity Induced in Colon by Chenodeoxycholate and Cholate

Intracolonic instillation		12-HETE	ODC
No. 1 ($t = 0$)	No. 2 ($t = 30$ min)	ng/g tissue	pmol CO_2 / min/mg protein
Saline	Saline	1.9 \pm 0.4	4.1 \pm 0.6
Phenidone	Saline	0.6 \pm 0.2*‡	3.8 \pm 0.6
Saline	Chenodeoxycholate (10 μmol)	3.8 \pm 0.5*	15 \pm 2*
Phenidone	Chenodeoxycholate (10 μmol)	0.9 \pm 0.2*‡	4.5 \pm 0.7‡
Saline	Cholate (20 μmol)	3.3 \pm 0.5*	12 \pm 2*
Phenidone	Cholate (20 μmol)	0.7 \pm 0.2*‡	3.5 \pm 0.7‡

Rats were treated with the test agents shown above as described in Table II legend. For determination of 12-HETE rats were killed 30 min after the second instillation. For determination of ODC, rats were killed 4 h after the second instillation. Results are means \pm SE of determinations on extracts of quick-frozen colon (12-HETE) or mucosal homogenates (ODC) of six rats in each group.

* $P < 0.01$ compared with corresponding value in rats receiving saline alone.

‡ $P < 0.01$ comparing phenidone values with corresponding value in rats receiving saline as the first instillation.

DNA synthesis also occurred in the normal proliferative zone, as shown for phenidone (Table V) and previously reported for indomethacin (1).

Bile salts likely represent one of several intraluminal factors that modulate proliferative activity of colonic epithelium (6–12). A physiologic role for bile salts in this process has been suggested by studies of Deschner and Raicht (6), which demonstrated a reduction in the proliferative activity of colonic epithelium of rats treated with a bile diversion procedure. Nevertheless, the mechanism by which bile salts initiate an increase in colonic epithelial proliferative activity is not known. To the extent that an increase in ODC represents an essential early step in this process, the action of bile salts to increase epithelial proliferation is not dependent upon the development of an inflammatory response. Thus, increased ODC at 4 h after deoxycholate was not associated with infiltration of inflammatory cells.

Deoxycholate and other bile salts have been shown to enhance PGE₂ and TXB₂ production by colon (5) (Tables II and III). However, it seems clear that enhanced PGE or TXB₂ production is not responsible for the stimulation of colonic mucosal ODC or DNA synthesis. Inhibition of PGE synthesis by indomethacin in colons not instilled with deoxycholate resulted in an increase in both mucosal ODC and [³H]Thd incorporation into mucosal DNA. Suppression by indomethacin of the increases in colonic PGE induced by deoxycholate was associated with an enhancement of the effects of deoxycholate on mucosal ODC and DNA synthesis. Moreover, inhibition of colonic thromboxane synthesis by UK 37,248 had no effect on basal or deoxycholate-induced increases in ODC or DNA synthesis (Tables II and III). Thus, it seems unlikely that colonic accumulation of either TXA₂, or its metabolite TXB₂, are involved in the acute modulation of colonic epithelial proliferation.

The present results strongly suggest that expression of the actions of deoxycholate and other bile salts to increase mucosal ODC and subsequent DNA synthesis involves bile salt-induced release of arachidonate and enhanced generation of lipoxygenase products, as measured by 12-HETE. It is of note that the relative potency of deoxycholate, chenodeoxycholate and cholate in the induction of colonic mucosal ODC observed in this and previous studies (8) correlates with their relative potency to stimulate the release of free arachidonate from colon (Fig. 1, deoxycholate > chenodeoxycholate > cholate). Increases in 12-HETE induced by deoxycholate were observed within 30 min, clearly preceded increases in ODC, and were not associated with histologic evidence of leukocytic infiltration. Thus, the 12-HETE likely was produced by cells indigenous to colon. The presence of 12-HETE has previously been described in extracts of mucosal and muscular layers of human colon (36). Phenidone, an inhibitor of both cyclooxygenase and lipoxygenase activity (26), suppressed bile salt-induced increases in mucosal ODC, and the increases in DNA synthesis induced by deoxycholate in the proliferative pool of colonic epithelial cells. The latter actions of phenidone correlated well with phenidone depression of colonic accumulation of 12-HETE.

The distinct effects of phenidone vs. indomethacin on bile salt-induced increases in mucosal ODC and DNA synthesis correlate best with the differential effects of these two agents on bile salt-induced increases in colonic accumulation of lipoxygenase products, as reflected by 12-HETE. In this regard, indomethacin inhibited basal and deoxycholate-induced increases in colonic PGE and malondialdehyde synthesis, but enhanced colonic 12-HETE accumulation. The increase in 12-HETE may reflect diversion of available free arachidonate from cyclooxygenase to lipoxygenase metabolism by indomethacin. Since exogenous PGE₂, the dominant PG product of colon (19, 37), inhibits colonic mucosal DNA synthesis *in vivo* and *in vitro* (1, 4), it seems likely that the increase in epithelial cell proliferative activity observed with indomethacin is related at least in part to inhibition of colonic PGE synthesis. However, it is possible that indomethacin-induced increases in 12-HETE also play a role in expression of the acute stimulation of basal colonic mucosal ODC and DNA synthesis by this agent, as well as the increases in these parameters induced by bile salts. Thus, indomethacin, which suppressed PGE but increased 12-HETE accumulation, stimulated proliferative activity. By contrast, phenidone, which inhibited both PGE and 12-HETE accumulation, abolished bile-salt induced increases in ODC and [³H]Thd incorporation into DNA. In contrast to its inhibition of the actions of bile salts, phenidone was without detectable effect on basal ODC or DNA synthesis of colonic epithelium. Whether more sustained inhibition of lipoxygenase activity in colon will lead to a decline in basal epithelial proliferative activity remains to be determined. The differential effects of the test agents used in the current study on colonic 12-HETE, basal, and deoxycholate-responsive proliferation of colonic epithelium are summarized in Table VII.

While accumulation of 12-HETE provides evidence for increased generation of 12-lipoxygenase products by colon exposed to bile salts, a role for 12-HETE in the expression of bile salt action on epithelial proliferation is not yet established. The profile of lipoxygenase products in colon and the capacity of various cellular components to generate these products remains to be established. It is known that oxidants are generated during metabolism of arachidonate by both the

Table VII. Differential Effects of Test Agents on Colonic Accumulation of 12-HETE and on Basal and Deoxycholate Responsive Proliferative Activity of Colonic Epithelium

Test agent	Colonic 12-HETE	Colonic epithelial proliferation	
		Basal	Deoxycholate responsive
Deoxycholate	↑	↑	—
Indomethacin	↑	↑	↑
Phenidone	↓	NC	↓
UK 37,248	NC	NC	NC

↑, stimulation; ↓, inhibition; NC, no detectable change.

cyclooxygenase and lipid peroxidation pathways (38, 39). These reactive moieties can interact with nucleic acids (40, 41) and other macromolecules in the cells, including membrane lipids, and thus may be involved in bile salt enhancement of colonic epithelial proliferative activity. Phenidone, a known antioxidant and free radical scavenger (41), could suppress bile salt-induced increases in proliferative activity by scavenging these oxidant radicals. Accordingly, inhibition of bile salt action on epithelial proliferation by phenidone may have been related to a general reduction in cellular peroxidative activity rather than the selective suppression of the formation of 12-lipoxygenase products. That phenidone indeed suppresses lipid peroxidative metabolism in colon is reflected by its inhibition of malondialdehyde formation by colon exposed to deoxycholate (Table II) (43). It is notable, however, that indomethacin also suppressed malondialdehyde formation, but stimulated epithelial proliferation. Therefore, a simple correlation between lipid peroxidation and epithelial proliferation does not exist.

In view of the inhibitory effect of exogenous PGE₂ on colonic mucosal DNA synthesis (1, 4), the basis for the failure of increases in colonic accumulation of endogenous PGE₂, induced by bile salts, to suppress proliferative activity of colonic epithelium remains speculative. Bile salts enhance the release of arachidonate and thus the generation of both cyclooxygenase and lipoxygenase products. The cellular sites of origin of these moieties could be critical determinants of the net change in epithelial proliferation observed. We have found that PGE₂ is generated predominantly in the submucosal regions of colon, and reaches the colonic mucosa intact in only limited quantities (19). Studies in guinea pig colon have indicated that lipoxygenase products may be preferentially formed in colonic mucosa relative to submucosal tissues (44). Thus, bile salts may lead to preferential exposure of colonic epithelium to lipoxygenase vs. cyclooxygenase products. Clearly, much remains to be learned about the actions of various cyclooxygenase and lipoxygenase products on colonic epithelial proliferation and the mechanisms by which these actions are expressed. However, the current results suggest that both cyclooxygenase and lipoxygenase products may play important and different roles in the local modulation of this process.

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