Effect of bile acids on electrical properties of rat colon: Evaluation of an *in-vitro* model for secretion

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SUMMARY A preparation of rat colon mucosa has been evaluated as a useful model to study effects of bile acids on the changes in short-circuit current and transmural electrical potential difference (pd) which others have associated with alterations in electrolyte transport. Using this preparation, it was found that bile acids were effective in increasing short-circuit current and pd when applied to the serosal, but not the mucosal, surface of the tissue. Furthermore, taurine-conjugated cholic acid, which has no demonstrable effect on the colon *in vivo*, was found to increase short-circuit current and pd significantly in the *in-vitro* preparation. These data indicate the limitations of the *in-vitro* model in studying the mechanism of bile acid-stimulated intestinal secretion.

The pathogenesis of diarrhoea caused by bile acids, termed cholerrhoeic enteropathy (Hofmann, 1967) or cholanorrhoeic diarrhoea (Hofmann, 1973), is imperfectly understood. Dihydroxy bile acids inhibited salt and water absorption and tended to initiate fluid secretion in studies on human and hamster jejunum (Teem and Phillips, 1972; Wingate, Phillips, and Hoffman, 1973) and human and canine colon (Mekhjian and Phillips, 1970; Mekhjian, Phillips, and Hofmann, 1971). In these studies, a trihydroxy bile acid (cholic acid) was without effect.

Binder and Rawlins (1973) used an *in-vitro* preparation of rat colonic mucosa to study Na and Cl transport, short-circuit current and transmural electrical potential difference (pd). They found that taurochenodeoxycholic acid, a taurine-conjugated dihydroxy bile acid, reduced net absorption of Na and Cl, and increased short-circuit current and pd: theophylline mimicked these effects and initiated secretion of Cl. They suggested that the *in-vitro* preparation demonstrated changes that occur when these bile acids are exposed to the colon *in vivo*, and may be responsible for the pathophysiological phenomena associated with bile acid diarrhoea.

Before data from this *in-vitro* model can be used to understand the changes seen *in vivo*, it is necessary

to determine what effect trihydroxy bile acid (cholic acid) may have when it is used. Cholic acid is without effect on jejunal and colonic salt and water absorption (Mekhjian and Phillips, 1970; Mekhjian *et al*, 1971; Wingate *et al*, 1973) and should be without effect *in vitro* if this model truly reflects *in-vivo* phenomena. Moreover, since bile acids are exposed *in vivo* to the mucosal surface of intestine, effects with the *in-vitro* model should occur when bile acids are exposed only to the mucosal surface of the preparation; in the study of Binder and Rawlins (1973) bile acids were applied to both the mucosal and serosal surfaces.

Since initiation of secretion by large and small intestine is accompanied by an increase in electrical activity of the tissue (Binder and Rawlins, 1973; Field, 1971), we measured changes in short-circuit current and pd of rat colon as an index of the state of secretory activity of the tissue. The taurine conjugates of the two primary bile acids, taurocholic acid and taurochenodeoxycholic acid, the taurine conjugate of deoxycholic acid, and theophylline were tested for effect at either the mucosal or the serosal surface.

Methods

Male Sprague-Dawley rats weighing 300-400 g were anaesthetized with ether and killed by intracardiac injection of 33 mg of pentobarbital. The proximal half of the colon was removed and washed with

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Ringer solution at 0°C. The serosa and part of the muscular layers were removed before mounting the tissue in the apparatus for measuring electrical properties as described previously (Rose, Gelarden, and Nahrwold, 1973). Briefly, 1.13 cm² of tissue was held between the halves of a Lucite chamber and exposed on each surface to 15 ml of buffered electrolyte solution at 37°C. The composition of the Ringer solution was (mM): NaCl, 140; MgCl₂, 1.2; CaCl₂, 1.2; K₂HPO₄, 1.2; KH₂PO₄, 0.2; KHCO₃, 10; and pH approximately 7.0. The bathing solution was circulated across each surface of the tissue by means of a gas-lift circulating system driven by water-saturated 95% O₂-5% CO₂.

Tips of Ringer-agar bridges were placed close to each surface of the membrane and the pd was measured using a pair of calomel electrodes leading to a high input impedance electrometer and recorded on a Beckman recorder. The tissue was short-circuited by applying external current; tissue resistance was determined by recording the pd deflection in response to 50 μ A of direct current from the external battery source.

The tissue was initially incubated in Ringer solution in the absence of theophylline and bile acids. After approximately 20 min, when the short-circuit current had stabilized, an appropriate volume of concentrated stock solution of bile acid (20 mM) or theophylline (100 mM) was added to one or both of the bathing solutions. The second compound was added to the existing solution 20-30 min after the first. When bile acid (final concentration, 2 mM) or theophylline (final concentration, 10 mM) was added only to the mucosal or the serosal bathing solution, mannitol was added to the other solution to maintain osmotic equilibrium. Bile acids were found to be at least 95% pure by thin-layer chromatography. Changes in short-circuit current and pd are reported as the difference between the recorded peak value after the addition of bile acid or theophylline and the value observed immediately before addition.

Results

BILE ACIDS

The addition of taurochenodeoxycholic acid (2 mM) to the mucosal and serosal bathing solutions of rat colon, or to the serosal solution alone, caused an increase in short-circuit current and pd which occurred within two min (fig 1A; table I). Applied to the mucosal surface alone, taurochenodeoxycholic acid was without effect (fig 1B). When taurochenodeoxycholic acid was added to the mucosal and/or serosal solution after the addition of 10 mM

theophylline there was no increase in short-circuit current. Similar results were obtained when taurodeoxycholic acid was used in place of taurochenodeoxycholic acid. Following inhibition of active transport processes and reduction of the transmural pd to zero by the application of cyanide (1 mM) or by anaerobic conditions, there was no effect on pd or short-circuit current due to taurodeoxycholic acid in both bathing solutions.

A significant decrease in tissue resistance was noticed with taurochenodeoxycholic acid.

Taurocholic acid (2 mM) added to the mucosal and serosal solutions of colon, or to the serosal solution alone, caused an increase in short-circuit current and pd (fig 1C, table II). The increase in short-circuit current seen when taurocholic acid was added to the serosal solution ($27 \pm 5 \mu$ A/cm²) was significantly smaller than the increase seen with taurochenodeoxycholic acid ($P < 0.05$). When taurocholic acid was added to the mucosal solution there was no effect on short-circuit current (fig 1D).

THEOPHYLLINE

Theophylline increased short-circuit current and pd whether applied to both surfaces of the colon or to

Fig 1 Representative experiments showing the effect of mucosal and serosal applications of taurochenodeoxycholic acid, taurocholic acid, and theophylline on pd of rat colon. Bile acid (2 mM) was added approximately 20 min after the tissue was mounted and theophylline (10 mM) was added after 40 minutes.

No	Theophylline Pretreatment	Initial Short-circuit Current ($\mu\text{A}/\text{cm}^2$)	Taurochenodeoxycholic Acid ¹	Δ PD (mv)	Δ Tissue Resistance ($\text{ohm}\cdot\text{cm}^2$)	Δ Short-circuit Current ($\mu\text{A}/\text{cm}^2$)
9	—	79 $\pm$ 12	Mucosal and serosal	2.4 $\pm$ 0.07 ^a	- 5 $\pm$ 2 ^a	42 $\pm$ 12 ^a
6	—	57 $\pm$ 13	Serosal	3.9 $\pm$ 0.6 ^a	-12 $\pm$ 2 ^a	81 $\pm$ 13 ^a
8	—	61 $\pm$ 10	Mucosal	0.1 $\pm$ 0.2	0 $\pm$ 1	1 $\pm$ 3
3	Mucosal and serosal ^a	109 $\pm$ 9	Mucosal and serosal	- 0.23 $\pm$ 0.03	0 $\pm$ 0	- 4 $\pm$ 2
5	Serosal	177 $\pm$ 27	Serosal	1.08 $\pm$ 0.57	- 2 $\pm$ 1	25 $\pm$ 12
3	Mucosal	140 $\pm$ 3	Mucosal	- 0.73 $\pm$ 0.53	- 2 $\pm$ 1	- 8 $\pm$ 1 ^a

Table I Effect of taurochenodeoxycholate on transmural pd, tissue resistance, and short-circuit current

¹Taurochenodeoxycholate was added to the mucosal and/or serosal solution at a final concentration of 2 mM.^aIndicates a statistically significant difference ($P < 0.05$) on the basis of a paired t test. Values reported are mean $\pm$ SEM. Sign of the pd change refers to the serosal solution. Changes in short-circuit current, pd, and resistance were calculated from the differences between the peak value after addition and the value immediately before the addition.^aTheophylline was added to the mucosal and/or serosal bathing solution at a final concentration of 10 mM.

No	Initial Short-circuit Current ($\mu\text{A}/\text{cm}^2$)	Taurocholate ¹	Δ PD (mv)	Δ Tissue Resistance ($\text{ohm}\cdot\text{cm}^2$)	Δ Short-circuit Current ($\mu\text{A}/\text{cm}^2$)
6	56 $\pm$ 6	Mucosal and serosal	1.3 $\pm$ 0.5 ^a	- 5 $\pm$ 2	23 $\pm$ 9 ^a
6	47 $\pm$ 10	Serosal	1.8 $\pm$ 0.3 ^a	- 3 $\pm$ 1	27 $\pm$ 5 ^a
4	66 $\pm$ 14	Mucosal	- 0.2 $\pm$ 0.1	- 2 $\pm$ 2	- 2 $\pm$ 2

Table II Effect of taurocholate on transmural pd, tissue resistance, and short-circuit current

¹Taurocholate was added to the mucosal and/or serosal solution at a final concentration of 2 mM. Other notation as in table I.

No	Pretreatment		Initial Short-circuit Current ($\mu\text{A}/\text{cm}^2$)	Theophylline ¹	Δ PD (mv)	Δ Tissue Resistance ($\text{ohm}\cdot\text{cm}^2$)	Δ Short-circuit Current ($\mu\text{A}/\text{cm}^2$)
	Mucosal Solution	Serosal Solution					
3	—	—	50 $\pm$ 9	Mucosal and serosal	4.2 $\pm$ 1.4 ^a	-15 $\pm$ 6	82 $\pm$ 18 ^a
5	—	—	45 $\pm$ 12	Serosal	7.4 $\pm$ 1.4 ^a	-17 $\pm$ 6 ^a	127 $\pm$ 18 ^a
3	—	—	30 $\pm$ 4	Mucosal	6.4 $\pm$ 1.1 ^a	-29 $\pm$ 10	112 $\pm$ 5 ^a
9	TCDC	TCDC	46 $\pm$ 9	Mucosal and serosal	- 0.05 $\pm$ 0.11	- 5 $\pm$ 1 ^a	7 $\pm$ 5
6	—	TCDC	39 $\pm$ 6	Serosal	- 0.03 $\pm$ 0.17	- 6 $\pm$ 1 ^a	5 $\pm$ 5
8	TCDC	—	25 $\pm$ 3	Mucosal	2.69 $\pm$ 0.61 ^a	-11 $\pm$ 3 ^a	57 $\pm$ 8 ^a
6	TC	TC	19 $\pm$ 3	Mucosal and serosal	2.23 $\pm$ 0.28 ^a	-19 $\pm$ 3 ^a	46 $\pm$ 8 ^a
6	—	TC	30 $\pm$ 7	Serosal	4.28 $\pm$ 0.33 ^a	-23 $\pm$ 5 ^a	78 $\pm$ 9 ^a
4	TC	—	18 $\pm$ 2	Mucosal	4.63 $\pm$ 1.18 ^a	-28 $\pm$ 6 ^a	104 $\pm$ 12 ^a

Table III Effect of theophylline on transmural pd, tissue resistance, and short-circuit current with or without pretreatment with bile acids

¹Theophylline was added to the mucosal and/or serosal solution at a final concentration of 10 mM. Other notation as in table I.

the mucosal or serosal surface alone (table III). When theophylline was added after the serosal application of taurochenodeoxycholic acid neither short-circuit current nor pd increased significantly (fig 1A). Following the mucosal application of taurochenodeoxycholic acid, theophylline had a stimulatory effect (fig 1B). Pretreatment with taurocholic acid at the mucosal and/or serosal surface reduced slightly the effect of theophylline.

Discussion

Binder and Rawlins (1973) found that in *in-vitro*

preparations of rat colonic mucosa taurochenodeoxycholic acid increased short-circuit current and pd simultaneously with decreased absorption of water and NaCl. We have confirmed their observation on electrical effects and found also that taurodeoxycholic acid and taurocholic acid are effective. The observation that the latter increased short-circuit current and pd is difficult to reconcile with observations *in vivo* that this bile acid does not alter water and electrolyte absorption.

The observation that bile acids acted when applied to the serosal surface, but not when applied to the mucosal surface, of the rat colon also makes inter-

pretation of the data from this *in-vitro* model difficult. Clearly, bile acids *in vivo* are exposed primarily to the mucosal surface of intestine, and any *in-vitro* action they have would be expected following mucosal, not serosal, application. Preliminary observations on rabbit ileum *in vitro* indicate that electrical phenomena associated with secretion can be produced when bile acids are applied to either the mucosal or serosal surface (Schwiter, Hepner, and Rose, unpublished observation). Thus, rabbit ileum may be a better model for future studies *in vitro*.

From the present and previous experiments (Binder and Rawlins, 1973) it appears that the increase in short-circuit current caused by conjugated dihydroxy bile acids reflects active transport of anions in the direction of secretion. Binder and Rawlins (1973) suggested that the effect of dihydroxy bile acids may be mediated by cyclic adenosine 3', 5'-monophosphate (cyclic AMP) and listed several effects of bile acids that are similar to those produced by theophylline as evidence. We have confirmed and extended these observations but a causal relationship between intracellular levels of cyclic AMP and secretion will remain a tentative conclusion until evidence is presented that cyclic AMP is stimulated at the time of the peak electrical and secretory response¹.

The transitory nature of the electrical response elicited by bile acids is not readily explained from information currently available. It is unlikely to be due to cell membrane damage causing temporary potassium leakage across the serosal cell surface since the response is immediately dependent on availability of O₂. A transitory electrical response was also observed in rat ileum *in vitro* exposed to taurocholic acid (Wall and Baker, 1974). In both tissues the response *in vitro* may be limited by intracellular accumulation of bile acids since a stable response was observed *in vivo* (Wall and Baker, 1974).

The recent observation by Wall and Baker (1974) that bile acids are effective in eliciting electrical responses in rat small intestine only when applied to the mucosal surface contrasts with our observation of an effect due to serosal application in colon. The small intestine preparation apparently was not stripped of serosa which might have prevented bile acids in the serosal bathing solution from reaching the transport cells.

¹While these studies were in progress a report appeared (Binder, H. J., Filburn, C., and Volpe, B. T. (1975). Bile salt alteration of colonic electrolyte transport: role of cyclic adenosine monophosphate. *Gastroenterology*, 68, 503-508) showing an increase in rat colonic mucosal cyclic AMP after incubation for 20 minutes with 2 mM taurochenodeoxycholic acid. However, we have been unable to confirm these observations (Wade, Hepner, and Rose, unpublished observations).

The effects on epithelial transport of such agents as theophylline, bile acids, and cyclic AMP might not represent a single change in tissue properties. The decreased tissue resistance in epithelial mucosa following exposure to bile acids could be the result of tissue damage. Thus, Frizzell and Schultz (1970) observed that protein is removed from the mucosal surface of rabbit ileum exposed to taurocholic acid, and Thomas, Nahrwold, and Rose (1972) found increased amounts of phospholipid in the lumen of rat stomach exposed to taurocholic acid. In addition, it has been found that theophylline, cyclic AMP, and cholera toxin inhibit unidirectional mucosa-to-serosa fluxes of Na and Cl across rabbit ileum by interfering with membrane components involved in transport (Frizzell, Nellans, Rose, Markscheid-Kaspi, and Schultz, 1973; Nellans, Frizzell, and Schultz, 1973). Thus, the final interpretation of experiments designed to study initiation of secretion must consider that alterations in transmural ionic fluxes might be the sum result of tissue destruction and inhibition of normal absorptive transport machinery as well as initiation of secretory processes.

There is currently considerable interest in using an animal model to study intestinal secretion associated with clinical diarrhoea caused by such agents as bile acids (Mekhjian and Phillips, 1970), fatty acids (Ammon and Phillips, 1974), and cholera toxin (Moore, Bieberdorf, Morawski, Finkelstein, and Fordtran, 1971). Previous reports have documented that dihydroxy bile acids in the concentrations used in the present experiments initiate electrolyte secretion by rat colonic mucosa. The present report may serve to inform concerned investigators that in the model of rat colon bile acids are effective in eliciting the electrical phenomena associated with secretion only when present at the serosal surface. The existing literature might require reinterpretation in view of this finding.

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