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BILE SALT ALTERATION OF ION TRANSPORT ACROSS JEJUNAL MUCOSA

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SUMMARY

The effect of conjugated dihydroxy and trihydroxy bile salts on electrolyte transport across isolated rabbit jejunal mucosa was studied. Both taurochenodeoxycholic acid and taurocholic acid increased the short-circuit current (I_{sc}) in bicarbonate-Ringer solution but not in a bicarbonate-free, chloride-free solution. Taurochenodeoxycholic acid was significantly more effective than taurocholic acid in increasing I_{sc}. The presence of theophylline prevented the taurochenodeoxycholic acid- and taurocholic acid-induced increase in I_{sc}. Transmural ion fluxes across jejunal mucosa demonstrated that 2 mM taurochenodeoxycholic acid decreased net Na^+ absorption, increased net Cl^- secretion and increased the residual flux (which probably represents HCO_3^- secretion). These studies support the hypothesis that cyclic AMP may be a mediator of intestinal electrolyte secretion.

INTRODUCTION

Bile salts affect jejunal absorptive function in both man and experimental animals [1–7]. Diminished absorption or net secretion of fluid and electrolytes and decreased monosaccharide absorption have been reported. Although bile salts may produce some morphological damage in the jejunum, many of these changes have been observed in the absence of any histologic abnormalities. A consistent observation in these previous studies is that dihydroxy bile salts have more profound effects on electrolyte transport than trihydroxy bile salts. Further, Wingate [6] observed that although perfusion of the jejunum with glycodeoxycholic acid resulted in net secretion, perfusion with a glycodeoxycholic acid/lecithin mixed micelle solution did not alter fluid movement.

The mechanism by which bile salts alter jejunal electrolyte transport has not been elucidated. These alterations of jejunal transport are qualitatively similar to those produced by bile salts on water and electrolyte movement in ileum and colon [8–10]. In previous in vitro studies which examined the effect of taurochenodeoxycholic acid and taurocholic acid on colonic ion movement, we have demonstrated that bile salts increase short-circuit current and have proposed that bile salt-induced

changes in ion transport may be mediated by the mucosal cyclic AMP [11, 12]. In this present study, we have studied the effect of bile salts on ion transport across isolated rabbit jejunum and have obtained results similar to our previous findings in the colon.

MATERIALS AND METHODS

Jejunal mucosa was obtained from non-fasting male white rabbits weighing approx. 2 lb. Following sacrifice, the pyloric area of the rabbits was isolated, the proximal small intestine was removed and the initial 45 cm discarded. The next 40 cm was used as jejunal mucosa. Methods which have been used in experiments of ileal and colonic mucosa were employed and have previously been described in detail [11, 13].

Briefly, the serosal and part of the muscular layers were removed and sheets of mucosa placed between Lucite chambers whose aperture measured 1.13 cm^2. Both sides of the tissue were bathed with solutions of identical ionic composition at 37 C by means of water-jacketed, gas-lift circulating reservoir systems. Two solutions, both at pH 7.4, were employed: a Ringer and an HCO_3^--free, Cl^--free solution. The composition of the Ringer solution in mmol/l was: Na^+, 140; K^+, 5.2; Mg^{2+}, 1.2; Ca^{2+}, 1.2; Cl^-, 119.8; HCO_3^-, 25; HPO_4^{2-}, 2.4; $H_2PO_4^-$, 0.4. Sodium isethionate, $CaSO_4$ and $MgSO_4$ were used in the HCO_3^--free, Cl^--free solution. The Ringer solution was oxygenated with O_2/CO_2 (95 : 5, v/v) and the HCO_3^--free, Cl^--free solution was 100 % O_2.

The spontaneous electrical potential difference (PD) across the tissue was monitored through agar bridges placed near each membrane surface. These bridges contained electrolyte solutions similar to that bathing the tissues and were connected to balanced calomel electrodes and a direct-reading potentiometer. The tissue was short-circuited continuously so that the PD was nullified by automatic voltage clamps except for periods of less than 30 s during which the spontaneous PD was recorded. 10–20 min following stabilization of both PD and I_{sc}, theophylline, bile salts or a mixed micelle of bile salts and lecithin was added to two or three of the tissues. All additions were made simultaneously to both the mucosal and serosal bathing solutions. Subsequent additions, wherein a bile salt was added to theophylline-pretreated tissue or theophylline to bile salt or mixed micelle-pre-treated tissue, were made 30 min after the initial addition. Preliminary control experiments demonstrated that, without any additions, PD and I_{sc} remained constant after initial stabilization for at least 60 min. Therefore, the effect of the addition of bile salt and theophylline was determined by the difference between the value observed immediately prior to the addition and the peak value recorded subsequent to the addition. The peak value occurred usually within 5–10 min following the addition.

In those experiments in which Na^+ and Cl^- fluxes ($J_{net}^{Na^+}$ and $J_{net}^{Cl^-}$, respectively) were determined, 2 mM taurochenodeoxycholic acid was present in both the mucosal and serosal bathing solutions prior to the start of the incubation period. Oppositely directed Na^+ fluxes using ^{22}Na and ^{24}Na were determined across the same piece of tissue and oppositely directed Cl^- fluxes using ^{36}Cl were determined across adjacent pieces of tissue as previously described [11, 13]. Net Na^+ and Cl^- movement was calculated from the difference between the two unidirectional fluxes ($J_{ms} - J_{sm}$ =

J_{net}). Results are expressed as μequiv/h per cm^2 and positive values represent net absorption; negative ones net secretion.

The purity of the bile salts employed was verified by thin-layer chromatography and was greater than 98 % in each instance. Taurocehenodeoxycholic acid and taurocholic acid were obtained from Calbiochem (LaJolla, Calif.). Theophylline was obtained from Nutritional Biochemical Co.; L-lecithin from Schwarz-Mann Co. (Orangeburg, N.Y.); ^{22}Na and ^{24}Na from I.C.N. Corp. (Irvine, Calif.) and ^{36}Cl from New England Nuclear Co. (Boston, Mass.).

All results are expressed as mean $\pm$ S. E. and standard statistical methods were employed.

RESULTS

Addition of bile salts

A stable I_{sc} and PD were observed for 60 min when jejunum was incubated in Ringer solution. The addition of 2 mM taurochenodeoxycholic acid to jejunal mucosa resulted in a prompt and sustained increase in I_{sc} (Table I). The mean peak increment in I_{sc} was 18 ± 3 $\mu A/cm^2$ and higher concentrations of taurochenodeoxycholic acid did not result in larger increments. In contrast to the results with taurochenodeoxycholic acid the addition of 2 mM taurocholic acid failed to cause a significant increase in I_{sc}. However, when higher concentrations of taurocholic acid were added, significant increments were noted. The addition of 6 mM taurocholic acid resulted in an increase of 19 ± 4 $\mu A/cm^2$.

Since an increase in I_{sc} may represent either an increase in cation movement from mucosa to serosa or an increase in anion movement from serosa to mucosa, further studies were designed to determine the origin of this increase in I_{sc}. HCO_3^-

TABLE I

EFFECT OF BILE ACIDS AND THEOPHYLLINE ON SHORT-CIRCUIT CURRENT IN RABBIT JEJUNUM: INFLUENCE OF THEOPHYLLINE, HCO_3^- AND Cl^-

Mean $\pm$ S.E.. Results are expressed as $\mu A/cm^2$ and represent the increase in I_{sc} observed following the addition of either conjugated bile acids or theophylline. This increment is obtained by the difference between the peak I_{sc} observed following the addition and the I_{sc} recorded immediately prior to the addition. HCO_3^- and Cl^- were replaced with isethionate in the HCO_3^--free, Cl^--free solution (see text for composition of the solutions). *n* represents the number of tissues studied.

Addition	Ringer solution				HCO_3^--free, Cl^--free solution	
	n	Theophylline absent	*n*	Theophylline present*	*n*	Theophylline absent
2 mM taurochenodeoxycholic acid	17	18 ± 3	4	0	6	2 ± 1
2 mM taurocholic acid	12	0	–	–	–	–
6 mM taurocholic acid	13	19 ± 4	4	0	4	1 ± 0.5
10 mM theophylline	30	36 ± 3	–	–	6	6 ± 4

* In these experiments, theophylline was added at 20 min and the bile acid was added 30 min later.

TABLE II

EFFECT OF 2 mM TAUROCHENODEOXYCHOLIC ACID ON ION FLUXES ACROSS RABBIT JEJUNUM

Mean ± S.E. All results expressed as μequiv/h per cm^2 except PD which is expressed as mV. n is number of tissues or tissue pairs studied. Unidirectional Na^+ fluxes were determined with ^{22}Na, ^{24}Na and Cl^- fluxes with ^{36}Cl. I_{sc} represents the time integral during the period of flux determination. J_R is the residual or unaccounted flux and is determined by $I_{sc} - J_{net}^{Na^+} - J_{net}^{Cl^-}$). In the control tissue J_R was 0.1 μequiv/h per cm^2 and in the taurochenodeoxycholic acid experiments 1.4 μequiv/h per cm^2. Positive values represent net absorption, negative ones net secretion. n.s., not significant.

	n	Muconal → serosal	Serosal → mucosal	Net	I_{sc}	PD (mV)
Sodium						
Control	12	6.6 ± 0.6	6.0 ± 0.8	+0.6 ± 0.7	1.4 ± 0.1	2.6 ± 0.1
2 mM taurochenodeoxycholic acid	12	5.7 ± 0.6	7.8 ± 0.9	−2.1 ± 0.7	1.9 ± 0.2	2.7 ± 0.1
P		n.s.	n.s.	< 0.02	< 0.05	n.s.
Chloride						
Control	27	6.3 ± 0.4	7.1 ± 0.4	−0.8 ± 0.6	1.6 ± 0.1	2.7 ± 0.1
2 mM taurochenodeoxycholic acid	15	6.7 ± 0.4	9.4 ± 0.6	−2.7 ± 0.7	2.1 ± 0.2	2.6 ± 0.2
P		n.s.	< 0.01	< 0.05	< 0.05	n.s.

and Cl^- were removed from the bathing reservoirs by substitution with isethionate. The addition of 2 mM taurochenodeoxycholic acid and 6 mM taurocholic acid to jejunal mucosa bathed in this HCO_3^--free, Cl^--free media failed to increase the I_{sc} (Table I).

Ion fluxes

Na^+ and Cl^- flux studies were performed across control and 2 mM taurochenodeoxycholic acid-treated jejunal mucosa (Table II). $J_{net}^{Na^+}$ in control tissues was 0.6 ± 0.7 μequiv/h per cm^2 and in bile salt-treated tissue was −2.1 ± 0.7 μequiv/h per cm^2. Net Cl^- secretion was significantly greater in the jejunum exposed to the bile salts than in control tissues. The unaccounted or residual flux calculated by

$$J_R = I_{sc} - (J_{net}^{Na^+} - J_{net}^{Cl^-})$$

probably represents bicarbonate transport [13–15]. A positive J_R is consistent with HCO_3^- secretion or H^+ absorption. In these studies, J_R was 0.1 μequiv/h per cm^2 in control tissue and was +1.4 μequiv/h per cm^2 in the jejunal mucosa exposed to taurochenodeoxycholic acid.

Addition of theophylline

The addition of 10 mM theophylline to jejunal mucosa bathed in Ringer solution also resulted in a significant increase in I_{sc} (Fig. 1), but when added to the HCO_3^--free, Cl^--free solution, only a minimal increase in I_{sc} was noted (Table I). Further, the addition of dibutyryl cyclic AMP to mucosa bathed in Ringer solution also resulted in an increase in I_{sc} of 19 ± 4 μA/cm^2. The addition of bile salts prior to the addition of theophylline or the addition of theophylline prior to the addition of bile

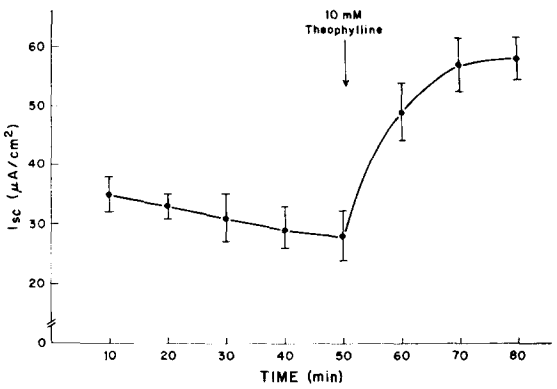

Fig. 1. Effect of 10 mM theophylline on I_{sc} in rabbit jejunum. The I_{sc} remained relatively stable during the initial 80 min of incubation. The increase in I_{sc} produced by the addition of either bile salts or theophylline at 20 and 50 min was determined by the difference between the peak I_{sc} observed immediately after and the I_{sc} present just prior to the addition. This figure demonstrates the effect of theophylline added to both mucosal and serosal solutions bathing jejunal mucosa not previously exposed to any bile salt.

salts markedly affected the increase in I_{sc} produced by theophylline and bile salts, respectively. As shown in Table I, when the mucosa was exposed to theophylline before 2 mM taurochenodeoxycholic acid or 6 mM taurocholic acid was added, no increase in I_{sc} was observed. The prior addition of bile salts similarly decreased by approx. 50 % the increment in I_{sc} observed following the addition of theophylline (Table I). Finally, the addition of taurochenodeoxycholic acid to mucosa previously exposed to dibutyryl cyclic AMP resulted in an increase in I_{sc} of only 4 ± 1 $\mu A/cm^2$.

TABLE III

EFFECT OF BILE SALTS AND THEOPHYLLINE ON SHORT-CIRCUIT CURRENT IN RABBIT JEJUNUM: INFLUENCE OF PHOSPHOLIPIDS

Mean ± S.E. All results expressed as $\mu A/cm^2$ represent the increase in I_{sc} calculated by the difference between peak I_{sc} observed after the addition of either bile salt or theophylline and that recorded immediately prior to the addition. Both the bile salt at approx. 20 min and theophylline 30 min later were added to mucosal and serosal solutions. n represents the number of tissues studied.

Bile salt	n	Increase in I_{sc} produced by bile salt	Increase in I_{sc} produced by theophylline after exposure to bile salt
None	30	—	36±3
2 mM taurochenodeoxycholic acid	17	18±3	18±3
2 mM taurochenodeoxycholic acid +1 mM lecithin	10	4±2	37±4
6 mM taurocholic acid	13	19±4	17±5
6 mM taurocholic acid+3 mM lecithin	11	6±3	29±9

Effect of mixed micelles

Although perfusion of human jejunum with dihydroxy bile salts results in water and Na^+ secretion, Wingate [5] has recently reported that absorption of water and electrolytes from a mixed micelle solution containing bile salts and phospholipids did not differ from non-bile salt-containing control solutions. Therefore, additional studies were performed to determine the effect of bile salts, when added as mixed micelles, on the electrical properties of intestinal mucosa. The results shown in Table III demonstrate the effect of the addition of bile salts with and without lecithin to jejunal mucosa. Although the addition of 2 mM taurochenodeoxycholic acid or 6 mM taurocholic acid to jejunal tissue increased I_{sc} and partially inhibited the subsequent increase in I_{sc} produced by the addition of theophylline, the addition of 2 mM taurochenodeoxycholic acid plus 1 mM lecithin or 6 mM taurocholic acid plus 3 mM lecithin produced significantly smaller increases in I_{sc} and failed to inhibit the increase in I_{sc} caused by theophylline.

Histology

Hematoxylin and eosin stain sections of jejunal mucosa incubated with taurochenodeoxycholic acid, taurochlic acid or theophylline did not reveal any significant morphological alterations compared to mucosa exposed to Ringer solution.

DISCUSSION

Forth et al. [1] initially demonstrated that bile salts affect jejunal water and electrolyte absorption. In these studies, unconjugated but not conjugated bile salts diminished absorption: net secretion was not observed. Several subsequent studies have consistently demonstrated net secretion of fluid and sodium. Further, dihydroxy bile salts appear more potent than trihydroxy bile salts in their alteration of sodium movement but conflicting evidence exists concerning the effect of conjugated bile salts on electrolyte transport.

Teem and Phillips [2] reported that, during continuous perfusions of hamster jejunum, conjugated deoxycholic acid and unconjugated chenodeoxycholic acid and deoxycholic acid produced water secretion. Conjugated forms of chenodeoxycholic acid and cholic acid and unconjugated cholic acid did not alter water absoption. No significant histologic damage was produced by conjugated bile salts. Sladen and Harries [3, 4] have observed similar results in experiments of rat jejunum with both closed loop and continuous perfusion methods.

Observations similar to those found in rodent jejunum have also been observed during human perfusion studies by Wingate [6] and Russel et al. [7]. In addition, Wingate [6] studied the effects of bile salt and mixed micelle solutions on fluid absorption. Net fluid and electrolyte secretion occurred during jejunal perfusion with a 2 mM glycodeoxycholic acid solution. In contrast, when a solution containing mixed micelles of glycodeoxycholic acid and lecithin was perfused, net absorption was demonstrated. These findings are consistent with the net fluid absorption that does occur in the proximal small intestine postprandially when bile salts and phospholipids are both present.

Our present in vitro studies confirm that bile salts alter jejunal electrolyte transport. Net secretion of both Na^+ and Cl^- was observed in the presence of tauro-

chenodeoxycholic acid. The addition of taurochenodeoxycholic acid resulted in an increase in I_{sc}. Although an increase in I_{sc} may represent either an increase in cation movement from mucosa to serosa or an increase in anion movement from serosa to mucosa, the results in Tables I and II suggest that the increase in I_{sc} is secondary to increased movement of Cl^- or HCO_3^- or both from serosa to mucosa. Removal of HCO_3^- and Cl^- prevented the bile salt-induced increase in I_{sc}, and flux studies demonstrated that taurochenodeoxycholic acid produced Na^+ and Cl^- secretion. Equimolar amounts of dihydroxy bile salts produced larger increments of I_{sc} than trihydroxy bile salts: chenodeoxycholic acid and deoxycholic acid are more potent inhibitors of fluid absorption in vivo than cholic acid. Additional correlation of in vivo and in vitro results was observed when the effects of addition of bile salts as either pure micelles or mixed micelles were compared. 2 mM taurochenodeoxycholic acid added alone increased I_{sc} by 18 $\mu A/cm^2$ but the addition of taurochenodeoxycholic acid and lecithin minimally increased I_{sc} (Table III).

Although in vivo studies have consistently demonstrated altered fluid and electrolyte movement n the presence of bile salts, they have not provided insight into the mechanism of bile salt action. Much emphasis has been placed on bile salt inhibition of sodium absorption. These in vitro studies demonstrate tha , in the presence of taurochenodeoxycholic acid, net Na^+ and Cl^- secretion is present. The methodology employed excludes increased hydrostatic or osmotic pressure, a change in intestinal motility or alteration in mucosal permeability as explanations for these events. These results are compatible with bile salt stimulation of NaCl secretion or bile salt inhibition of NaCl absorption associated either with a pre-existing secretory process or with stimulation of a secretory process. It is not completely possible to distinguish between these two possibilities. However, indirect evidence would favor bile salt stimulation of a secretory process.

Our previous observations in rat colon have suggested that bile salt alteration of ion transport is mediated by mucosal cyclic AMP [11, 12]. In these present studies, theophylline increased I_{sc}. More important, the presence of either theophylline or dibutyryl cyclic AMP inhibited the increase in I_{sc} produced by taurochenodeoxycholic acid. Conversely, the presence of taurochenodeoxycholic acid inhibited the increase in I_{sc} normally produced by theophylline (Table I). These observations are consistent with an increase in jejunal mucosal cyclic AMP by taurochenodeoxycholic acid although they do not prove that bile salt alteration of ion transport is directly secondary to bile salt augmentation of mucosal cyclic AMP.

Bile salts are surface active agents and the effect of bile salts on electrolyte transport may represent bile salt action at a surface membrane locus. The difference between dihydroxy and trihydroxy bile salts may be a manifestation of surface events: trihydroxy bile salts are more polar than dihydroxy bile salts and, therefore, mucosal entry of dihydroxy bile salts occurs more rapidly than that of trihydroxy bile salts. Enlargement of the micelle by the addition of lecithin results in a decrease in bile salt absorption. Schiff et al. [16] have suggested that the rate of diffusion across the unstirred water layer is rate limiting for mixed micelle absorption. Our results can be explained if the diminished diffusion of the enlarged mixed micelle results in a lower concentration of bile salts at the water-lipid interface and if this site is the locus of bile salt action.

We have already reviewed the evidence to support the concept that bile salts

may increase mucosal cyclic AMP. Mucosal cyclic AMP production is catalyzed by adenylate cyclase, a mucosal enzyme, and cholera enterotoxin which is also known to stimulate secretion by increasing mucosal cyclic AMP, interacts with a receptor of the cell membrane [17, 18]. These studies, therefore, are consistent with, but do not prove that, bile salts stimulate adenylate cyclase which then increases mucosal cyclic AMP. These studies demonstrate that bile salts produce secretion in vitro and provide indirect evidence that this secretion is secondary to bile salt stimulation of a cyclic AMP-mediated process.

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