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Ursodeoxycholic acid action on the transport function of the small intestine in normal and cystic fibrosis mice

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Abstract

Ursodeoxycholic acid possesses choleretic and cytoprotective properties and in cystic fibrosis (CF) it is used to treat the hepatobiliary symptoms of the disease. This study investigated the effects of this bile acid on the transport function of the small intestine in normal and CF mice. The effects of ursodeoxycholic acid were monitored as changes in short-circuit current (SCC) in stripped sheets of small intestine from normal (Swiss MF1) and transgenic CF (*Cftr*^{tm2Cam}) mice. In ileal sheets from Swiss MF1 mice, mucosal ursodeoxycholic acid caused a biphasic increase in SCC. The first phase was reduced by lowering the mucosal Na^+ concentration, while the second phase was inhibited by Cl^--free conditions, serosal furosemide or mucosal diphenylamine-2-carboxylic acid (DPC), suggesting an initial Na^+-dependent bile acid absorption followed by a stimulation of electrogenic Cl^- secretion. Serosal application of ursodeoxycholic acid to the ileum and mucosal or serosal application to the mid-intestine and jejunum elicited a secretory response only. Secretion was Ca^{2+}-dependent, but did not involve neural mechanisms. Mucosal mast cells, histamine and serotonin (5-HT) were implicated in the secretory response. Responses in tissues from transgenic wild-type mice were similar to those obtained with Swiss MF1 mice, but the secretory response to mucosal or serosal application of the bile acid was impaired in CF tissues. In ilea from CF mice the initial absorptive phase of the response to mucosal ursodeoxycholic acid was still observed. It is concluded that ursodeoxycholic acid induces secretion throughout the murine small intestine by a mechanism that involves degranulation of mucosal mast cells. In the ileum Na^+-dependent absorption can also be detected. The secretory response is defective in CF intestine, but the absorptive effect is still present.

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Introduction

Ursodeoxycholic acid is a tertiary bile acid that is common in bear bile, but is only present in human bile in trace amounts. It is, however, used therapeutically for its choleretic and cytoprotective properties in the treatment, for example, of primary biliary cirrhosis and gallstones (Hofmann 1998). It can then account for as much as 40% of the total bile acid pool. In cystic fibrosis (CF), liver disease occurs in an increasing number of patients. Ursodeoxycholic acid can be used to treat the hepatobiliary symptoms of CF (Cheng et al 1997) and has been shown to normalize liver function tests and prevent the progression of fibrosis (Lindblad et al 1998).

Ursodeoxycholic acid is administered orally in doses (10–20 mg kg^{-1} daily) that lead to significant concentrations in the lumen of the gastrointestinal tract. It is known that bile acids can cause intestinal secretion (Binder 1980) and ursodeoxycholic acid has been shown to share this effect in rabbit ileum (Fasano et al 1994), although not in rabbit jejunum (Fasano et al 1994) or in mouse colon

(Gelbmann et al 1995). The mechanism responsible for the effect of ursodeoxycholic acid on the transport function of the intestine has not been systematically investigated. Neither has the action of this bile acid on intestinal transport in CF been examined. The development of transgenic CF mouse models has provided an opportunity to study the transport activity of CF intestine (Grubb & Gabriel 1997) and the aim of this investigation was to characterize the action of ursodeoxycholic acid in normal intestine and to compare this with its effect in CF tissues.

Materials and Methods

Chemicals

The following drugs were used: acetylcholine chloride, atropine sulfate, chenodeoxycholic acid, cholic acid, deoxycholic acid, dimethyl sulfoxide (DMSO), ethylene glycol-bis-(β-amino-ethyl) *N*,*N*′-tetra-acetic acid (EGTA), furosemide (frusemide), glycocholic acid, 5-hydroxytryptamine creatinine sulfate (5-HT), indometacin, sodium cromoglicate, substance P, taurocholic acid, tetrodotoxin, ursodeoxycholic acid (Sigma Chemical Company Ltd, Poole, UK), diphenylamine-2-carboxylic acid (DPC, *N*-phenylanthranilic acid; Fluka Chemicals, Gillingham, UK), doxantrazole (3-(1*H*-tetrazol-5-yl)-9*H*-thioxanthen-9-one-10,10-dioxide monohydrate (Aldrich Chemical Company, Poole, UK), mepyramine maleate, mannitol (May & Baker, Dagenham, UK), glucose (Fisons Scientific Equipment, Loughborough, UK). Other chemicals were of analytical grade and obtained from commercial suppliers.

Animals

Experiments were performed on intestinal tissues from mice killed by cervical dislocation in accordance with UK Home Office regulations and with local ethical committee approval. Male Swiss MF1 mice (age 12–13 weeks, body weight 20–30 g) were obtained from the Sheffield Field Laboratories. In the transgenic cystic fibrosis (CF) mouse model used (*Cftr*$^{tm\,2Cam}$) the $\triangle$F508 mutation was introduced into the CFTR gene (Colledge et al 1995). Transgenic mice were bred in the Sheffield Field Laboratories and animals used in the study included mice homozygous for the $\triangle$F508 mutation together with wild-type littermates. All mice were allowed free access to food and water.

Measurement of transintestinal electrical activity

The potential difference (PD), short-circuit current (SCC) and tissue resistance were measured across paired sheets of small intestine. Most tissues were stripped of their outer muscle layers and myenteric plexus, but in a few experiments intact sheets were used. The ileum was taken from the region immediately adjacent to the caecum, the mid-intestine from the mid region and the jejunum from the region just distal to the ligament of Treitz. Each sheet was mounted in an Ussing chamber with an aperture of 0.5 cm^2 and incubated at 37°C in Krebs bicarbonate saline gassed with 95% O_2—5% CO_2. The serosal fluid contained 10 mM glucose and the mucosal fluid 10 mM mannitol and each had a volume of 5 mL. The PD was measured using salt-bridge electrodes connected via calomel half cells to a differential input electrometer with output to a two-channel chart recorder (Linseis L6512). Current was applied across the tissue via conductive plastic electrodes and tissue resistance was determined from the PD change induced by a 50-μA current pulse, taking into account the fluid resistance. The SCC generated by the sheets was calculated from PD and resistance measurements using Ohm's law.

Tissues were allowed to stabilize for 15 min after mounting and then readings of electrical activity were taken at 1-min intervals. Following 5 min of basal readings, bile acid was added to the preparation and readings were continued for a further 10 min.

Normal Krebs bicarbonate saline contained (in mM): Na^+, 143.4; K^+, 5.9; Ca^{2+}, 2.5; Mg^{2+}, 1.2; Cl^-, 125.7; HCO_3^-, 24.9; $H_2PO_4^-$, 1.2; SO_4^{2-}, 1.2. In Cl^--free conditions all Cl^- in both mucosal and serosal solutions was replaced with gluconate; in Ca^{2+}-free conditions serosal $CaCl_2$ was replaced with equimolar NaCl and 0.5 mM EGTA was added; the mucosal Na^+ concentration was reduced to 25 mM by replacing all NaCl with isotonic mannitol.

With the exception of taurocholic acid, which was dissolved in 154 mM NaCl, bile acids were dissolved in ethanol and in each case 100 μL stock solution was added to 5 mL bathing solution. Neither NaCl nor ethanol, added mucosally or serosally, had any significant effect on basal electrical activity.

Inhibitors were added to test sheets as soon as the preparations were set up, with control sheets receiving an equivalent volume of vehicle. Vehicles used to prepare stock solutions were: furosemide, DMSO; DPC, ethanol; and in each case 25 μL stock solution was added to 5 mL bathing solution. Indometacin was dissolved in

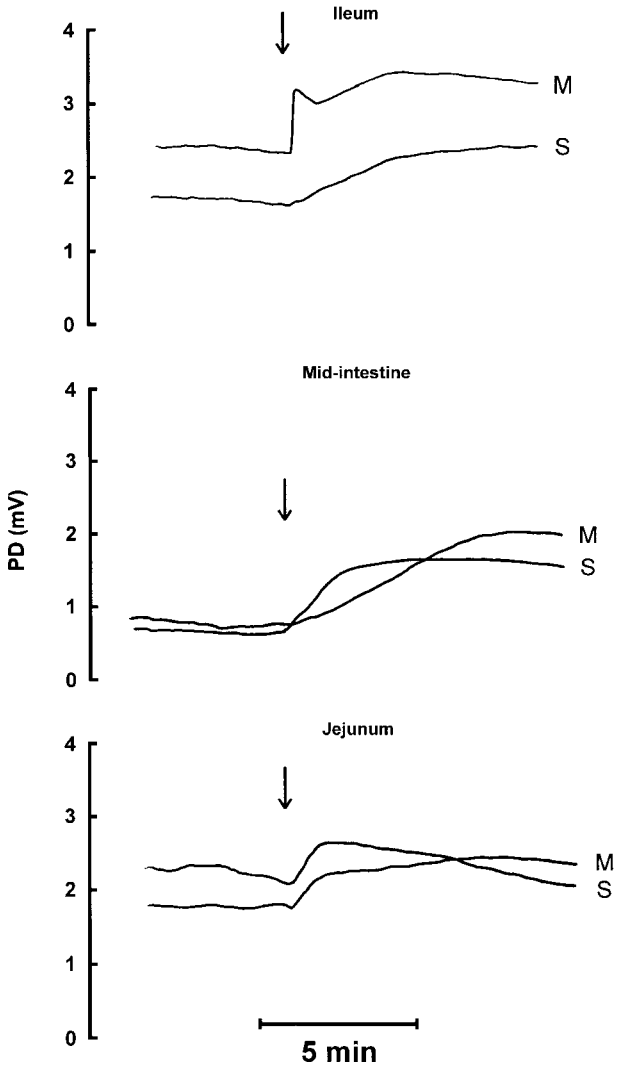

Figure 1 Typical responses of stripped sheets of mouse ileum, mid-intestine and jejunum to ursodeoxycholic acid. The PD is displayed as a function of time and 1 mM ursodeoxycholic acid was added to the mucosal (M) or serosal (S) solution at the point indicated by the arrow.

10% ethanol in 0.2% Na_2CO_3 and 100 μL was added to the serosal solution. None of the vehicles affected the response to ursodeoxycholic acid ($P > 0.05$ in all cases). All other drugs were dissolved in 154 mM NaCl and 100 μL was added to 5 mL bathing solution. Drugs were added to the serosal solution, except DPC (mucosal addition), and doxantrazole and sodium cromoglicate (mucosal + serosal addition).

Desensitization to 5-HT was achieved by adding 10^{-4} M 5-HT to the serosal solution (100 μL 154 mM NaCl in control) after 5 min of basal readings and testing the effect of ursodeoxycholic acid 10 min later. Preliminary experiments indicated that this protocol completely eliminated a second response to 10^{-4} M 5-HT (control: 83.8 ± 9.7 μA cm^{-2}; test: -6.0 ± 4.5 μA cm^{-2}, n = 7, $P < 0.001$). A similar protocol was adopted for desensitization to substance P. The first application of 10^{-6} M substance P increased the SCC by 29.6 ± 7.5 μA cm^{-2} (n = 9), but a second application was without effect (-0.2 ± 0.2 μA cm^{-2}, $P < 0.05$).

To test the selectivity of the effects of different experimental conditions, glucose (10 mM) was added to the mucosal solution of separate sheets after 5 min of basal readings. Under control conditions, glucose increased the SCC by 248.1 ± 11.7 μA cm^{-2} (n = 41).

The phenotype of the CF mice was confirmed using one pair of sheets from the mid-intestine in which acetylcholine (10^{-3} M) was added to the serosal solution after 5 min of basal readings, with glucose (10 mM) being added to the mucosal solution 5 min later to confirm tissue viability. Ursodeoxycholic acid action was tested in a second pair of sheets from the mid-intestine and in a pair of ileal sheets.

Measurement of fluid transport

Fluid uptake was measured in everted sacs of small intestine. Paired sacs of mid-intestine, each 5 cm long, were filled with approximately 0.2 mL Krebs bicarbonate saline containing 10 mM glucose and incubated for 30 min in 15 mL Krebs bicarbonate saline containing 10 mM mannitol at 37°C in a shaking water bath (80 oscillations per min). One of each pair was exposed to mucosal ursodeoxycholic acid (1 mM) and the other sac to an equivalent volume (0.67% v/v) of the ethanol vehicle. Ileal sacs were prepared similarly, but only one sac was taken from each mouse. Fluid uptake was determined as the increase in the weight of the filled sac during incubation and was related to the initial wet weight (iww) of the empty sac. The effects of furosemide were tested by adding it to the serosal fluid at a concentration of 10^{-3} M.

Expression of results

Results are expressed as mean values $\pm$ 1 s.e.m. of the number of observations indicated. Student's *t*-test, paired or unpaired as appropriate, was used to assess significance.

Results

Tissues from Swiss MF1 mice were used to characterize the intestinal transport responses to ursodeoxycholic acid, and the effects of cystic fibrosis on these processes were then investigated in intestinal sheets from transgenic CF mice.

Swiss MF1 mice

All regions of the intestine generated a basal PD and SCC in which the serosal side of the tissue was positive with respect to the mucosal side. In stripped ileal sheets, basal PD, SCC and tissue resistance values were 2.0 ± 0.1 mV, 63.2 ± 2.4 μA cm^{-2} and 31.6 ± 0.6 Ω cm^2 (n = 92). Corresponding values were 0.8 ± 0.1 mV, 30.9 ± 2.2 μA cm^{-2} and 26.7 ± 0.7 Ω cm^2 (n = 83) in stripped mid-intestine and 1.7 ± 0.1 mV, 61.7 ± 4.3 μA cm^{-2} and 28.1 ± 1.4 Ω cm^2 (n = 23) in stripped jejunum.

Effects of ursodeoxycholic acid on transintestinal electrical activity

Addition of 1 mM ursodeoxycholic acid to the mucosal solution of ileal sheets caused a biphasic increase in the PD and SCC (Figure 1, Table 1). There was an initial transient peak followed by a more gradual rise. Serosal application of the bile acid also increased the SCC, but in this case the effect was monophasic and the time taken to reach the maximum was longer. In the mid-intestine and jejunum both mucosal and serosal application of 1 mM ursodeoxycholic acid caused monophasic rises in PD and SCC (Figure 1, Table 1). The magnitude of the response to mucosal and serosal ursodeoxycholic acid was similar in the mid-intestine, but in the jejunum the response was smaller when the bile acid was added to the serosal compartment. In both regions the peak SCC was achieved more rapidly when ursodeoxycholic acid was added serosally. Ursodeoxycholic acid did not reduce tissue resistance in any of the regions of small intestine tested.

The concentration dependency of ursodeoxycholic acid action was examined in the ileum and mid-intestine (Figure 2). EC50 (concentrations causing 50% maximum response) values for the first and second phases of the ileal response to mucosal ursodeoxycholic acid were 0.12 mM and 0.13 mM, respectively. For the mid-intestine a value of 0.42 mM was obtained. Serosal application of the bile acid generated EC50 values of 0.17 mM in the ileum and 0.24 mM in the mid-intestine.

A second application of 1 mM ursodeoxycholic acid to the mucosal solution 10 min after the first did not elicit any increase in SCC in either the ileum or mid-intestine (Table 2), indicating that desensitization had occurred. However, a partial response was seen if the initial application of the bile acid was washed out after 10 min and then a second application made after a further 15 min. This was 46.0 ± 6.6% and 25.8 ± 4.9% of the initial responses for the first and second phases in the ileum (n = 6) and 43.0 ± 3.2% in the mid-intestine (n = 5).

Effects of other bile acids

The effects of other bile acids on transintestinal electrical activity were compared with those of ursodeoxycholic acid (Table 3). In the ileum, mucosal application of taurocholic, deoxycholic, chenodeoxycholic, glyco-

Table 1 Effect of ursodeoxycholic acid on the SCC in stripped sheets of mouse small intestine.

	Mucosal			Serosal	
	M1 (μA cm^{-2})	M2 (μA cm^{-2})	t (min)	S (μA cm^{-2})	t (min)
Ileum	31.6 ± 1.1 (92)	55.2 ± 1.8 (92)	3.7 ± 0.1 (92)	32.3 ± 3.3*** (15)	5.5 ± 0.5*** (15)
Mid-intestine	–	37.9 ± 1.7 (83)	7.8 ± 0.1 (83)	41.2 ± 4.6 (15)	5.7 ± 0.4*** (15)
Jejunum	–	32.5 ± 4.4 (23)	6.7 ± 0.5 (23)	12.1 ± 2.8* (8)	3.8 ± 0.6** (8)

Ursodeoxycholic acid (1 mM) was added to either the mucosal or serosal solution. M1 represents the rapid initial phase of the biphasic ileal response to mucosal application and M2 the second phase. S is the response to serosal application and t denotes the time taken to achieve the maximum SCC. Each value represents the mean $\pm$ 1 s.e.m. of the number of observations in parentheses. An unpaired *t*-test was used to compare the effects of mucosal (M2) and serosal application of ursodeoxycholic acid. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

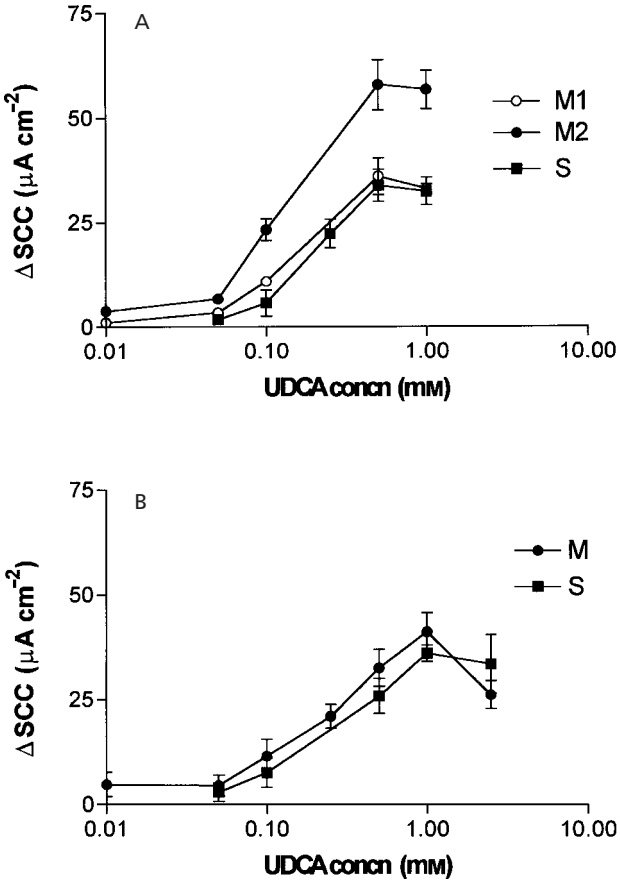

Figure 2 Concentration dependence of ursodeoxycholic acid (UDCA) action in stripped sheets of mouse ileum (A) and mid-intestine (B). Ursodeoxycholic acid was added to either the mucosal (M) or serosal (S) solution with M1 and M2 representing the first and second phase, respectively, of the ileal response. Each point represents the mean $\pm$ 1 s.e.m. of 4–15 observations.

cholic and cholic acids each caused an initial rise in SCC, but only taurocholic and glycocholic acids caused a secondary increase. In contrast, serosal addition of taurocholic, glycocholic and cholic acids had little effect, but deoxycholic acid and chenodeoxycholic acid both increased the SCC.

In the mid-intestine and jejunum, ursodeoxycholic acid was most effective in eliciting a response to mucosal application, with the other bile acids having little or no effect (Table 3). When added serosally, ursodeoxycholic, deoxycholic, chenodeoxycholic, glycocholic and cholic acids all caused a rise in SCC. Serosal taurocholic acid was without effect in the mid-intestine, but caused a large response in the jejunum. There are, therefore, regional variations in the intestinal response to bile acids.

Ionic basis of the SCC response to ursodeoxycholic acid

The first phase of the ileal response to mucosal ursodeoxycholic acid was inhibited when the Na^+ concentration of the luminal solution was reduced to 25 mM (Figure 3). These conditions also inhibited the rise in SCC induced by 10 mM glucose (control: 224.2 ± 27.7 μA cm^{-2}; low Na^+: 98.5 ± 15.9 μA cm^{-2}, n = 8, $P < 0.001$), but not the response to 10^{-3} M acetylcholine (control: 210.2 ± 21.7 μA cm^{-2}; low Na^+: 206.5 ± 28.8 μA cm^{-2}, n = 4, $P > 0.05$). The first part of the ursodeoxycholic acid response was not, however, affected by conditions that inhibit electrogenic Cl^- secretion. In contrast, the second phase of the ileal response was unaffected by a low mucosal Na^+ concentration, but it was reduced by conditions that inhibit electrogenic Cl^- secretion (Figure 3). These included removal of mucosal and serosal Cl^-, addition of furosemide (inhibits the Na^+,K^+,2Cl^- cotransporter responsible for Cl^- accumulation within the enterocyte; Heintze et al 1983) to the serosal solution and addition of DPC (blocks luminal Cl^- channels; Sahi et al 1994) to the mucosal solution. None of these conditions affected the response to 10 mM glucose ($P > 0.05$, n = 4 in all cases). The response to serosal ursodeoxycholic acid was also inhibited by furosemide (control: 26.6 ± 2.6 μA cm^{-2}; furosemide: 7.7 ± 3.0 μA cm^{-2}, n = 7, $P < 0.01$), as was the response to 10^{-3} M acetylcholine (control: 214.4 ± 36.4 μA cm^{-2}; furosemide: 90.9 ± 21.3 μA cm^{-2}, n = 4, $P < 0.05$).

In the mid-intestine the response to mucosal ursodeoxycholic acid exhibited the same characteristics as the second phase of the ileal response (Figure 3), in that it was unaffected by low mucosal Na^+, but reduced by conditions that inhibit electrogenic Cl^- secretion. Similarly, the effect of serosal ursodeoxycholic acid was inhibited by furosemide (control: 37.9 ± 6.8 μA cm^{-2}; furosemide: 3.0 ± 2.0 μA cm^{-2}, n = 8, $P < 0.01$).

The jejunal response to mucosal ursodeoxycholic acid (1 mM) was inhibited by both Cl^--free conditions (control: 38.7 ± 5.5 μA cm^{-2}; Cl^--free: 6.1 ± 2.0 μA cm^{-2}, n = 7, $P < 0.01$) and furosemide (control: 38.2 ± 8.1 μA cm^{-2}; furosemide: 5.3 ± 9.2 μA cm^{-2}, n = 8, $P < 0.05$).

The first phase of the ileal response to mucosal ursodeoxycholic acid appears to reflect Na^+-dependent absorption of the bile acid, while the second phase represents electrogenic Cl^- secretion. The response to mucosal application in the mid-intestine and jejunum and to serosal application in all regions is consistent with a stimulation of electrogenic Cl^- secretion. The mechanisms involved in the secretory response to muco-

Table 2 Reversibility of ursodeoxycholic acid action in stripped sheets of mouse small intestine.

		1st Application		2nd Application	
	n	M1 (μA cm^{-2})	M2 (μA cm^{-2})	M1 (μA cm^{-2})	M2 (μA cm^{-2})
Ileum (no wash)	5	28.3 ± 3.5	63.1 ± 6.7	0.8 ± 0.9**	−13.1 ± 2.6***
Ileum (wash)	6	25.4 ± 3.8	65.9 ± 5.8	12.9 ± 1.9*	19.7 ± 3.6***
Mid-intestine (no wash)	6	–	35.3 ± 3.8	–	−8.5 ± 8.4***
Mid-intestine (wash)	5	–	52.8 ± 6.4	–	33.2 ± 3.8**

Ursodeoxycholic acid (1 mM) was added to the mucosal solution and in the first experiment (no wash) a second application was made 10 min later. In the second experiment (wash) the first addition of ursodeoxycholic acid was washed out after 10 min and the second addition was made after a further 15 min. In control sheets the first addition was 100 μL ethanol and the second was 1 mM ursodeoxycholic acid, whose effects did not differ significantly ($P > 0.05$ in all cases) from those of the first bile acid addition to test sheets. M1 represents the rapid initial phase of the biphasic ileal response to mucosal ursodeoxycholic acid and M2 the second phase. Each value represents the mean ± 1 s.e.m. of the number of observations (n) indicated. A paired *t*-test was used to compare the effects of the first and second applications of ursodeoxycholic acid. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 3 Comparison of the effects of bile acids on the SCC in stripped sheets of mouse small intestine.

		Mucosal		Serosal
		M1 (μA cm^{-2})	M2 (μA cm^{-2})	S (μA cm^{-2})
Ileum	UDCA	35.0 ± 4.7 (8)	49.2 ± 5.8 (8)	37.4 ± 5.2 (8)
	TA	35.0 ± 5.1 (6)	92.7 ± 8.5 (6)	1.6 ± 2.0 (4)
	DCA	38.2 ± 4.5 (5)	7.2 ± 6.4 (5)	31.5 ± 3.9 (5)
	CDCA	28.8 ± 5.6 (6)	1.9 ± 5.2 (6)	27.9 ± 2.0 (5)
	GCA	25.1 ± 3.3 (5)	40.8 ± 5.3 (5)	7.2 ± 3.1 (5)
	CA	29.2 ± 5.0 (7)	12.3 ± 3.3 (7)	3.1 ± 3.3 (7)
Mid-intestine	UDCA	–	41.6 ± 4.9 (7)	45.0 ± 6.3 (7)
	TA	–	9.4 ± 4.7 (6)	3.6 ± 1.2 (6)
	DCA	–	−4.1 ± 1.8 (8)	63.8 ± 7.7 (4)
	CDCA	–	7.6 ± 4.0 (6)	62.0 ± 5.3 (6)
	GCA	–	10.5 ± 4.5 (6)	16.9 ± 3.0 (6)
	CA	–	7.8 ± 3.4 (6)	18.9 ± 2.0 (6)
Jejunum	UDCA	–	21.3 ± 7.9 (8)	12.1 ± 2.8 (8)
	TA	–	7.8 ± 1.7 (7)	96.7 ± 20.1 (8)
	DCA	–	−9.9 ± 6.5 (8)	54.6 ± 7.9 (8)
	CDCA	–	4.7 ± 6.4 (8)	48.8 ± 5.9 (8)
	GCA	–	0.2 ± 0.8 (4)	12.6 ± 2.3 (4)
	CA	–	11.5 ± 4.4 (7)	44.7 ± 8.9 (7)

Ursodeoxycholic acid (UDCA, 1 mM), taurocholic acid (TA, 1 mM), deoxycholic acid (DCA, 1 mM), chenodeoxycholic acid (CDCA, 1 mM), glycocholic acid (GCA, 1 mM) or cholic acid (CA, 1 mM) were added to either the mucosal or serosal solution. M1 and M2 represent the first and second phases of the ileal response to mucosal application and S is the response to serosal application. Each value represents the mean ± 1 s.e.m. of the number of observations in parentheses.

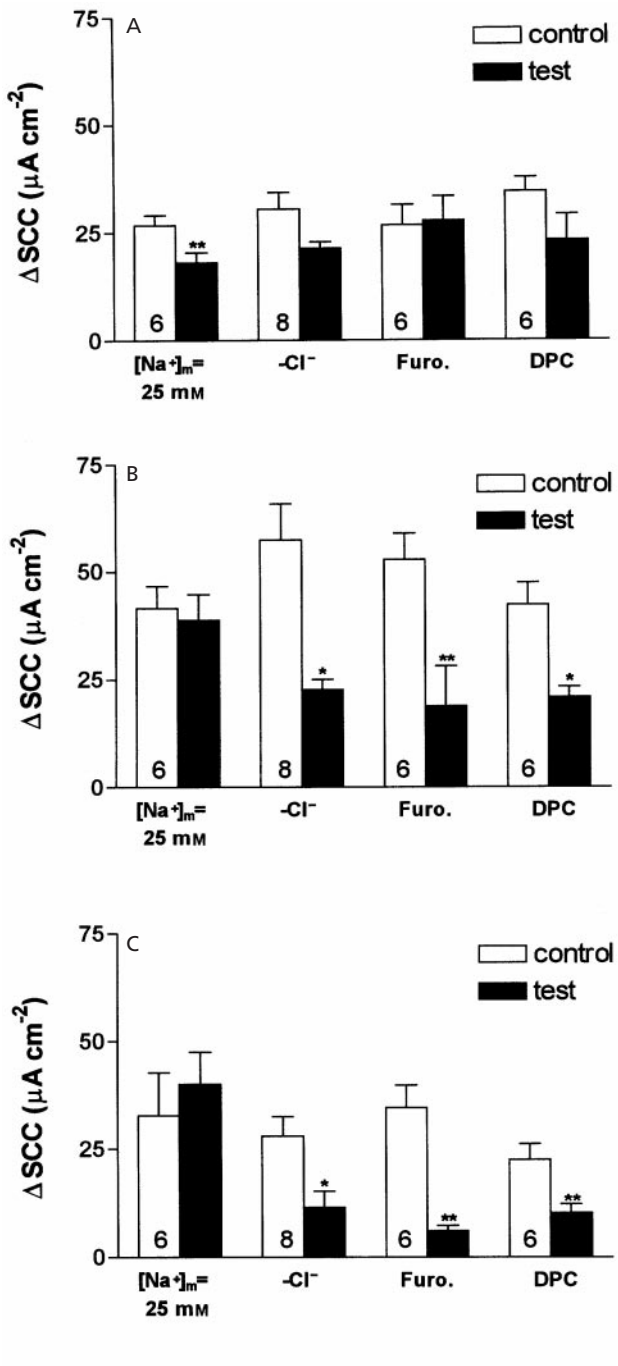

Figure 3 Ionic basis of the SCC response of stripped sheets of mouse ileum and mid-intestine to ursodeoxycholic acid. The effects of reducing the mucosal Na^+ concentration to 25 mM ($[Na^+]_m$ = 25 mM), absence of mucosal and serosal Cl^- ($-Cl^-$), 10^{-3} M serosal furosemide (Furo.) and 5×10^{-4} M mucosal diphenylamine-2-carboxylic acid (DPC) on the first (A) and second (B) phase in the ileal response and the mid-intestinal response (C) to 1 mM mucosal ursodeoxycholic acid are shown. Where appropriate, control sheets received an equivalent volume of vehicle. Each bar represents the mean $\pm$ 1 s.e.m. of the number of tissue pairs indicated in the columns and a paired *t*-test was used to assess significance: $*P < 0.05$; $**P < 0.01$.

sal ursodeoxycholic acid were examined in more detail in the ileum and mid-intestine.

Involvement of Ca^{2+} and neural pathways in ursodeoxycholic acid action

The secretory phase of the ursodeoxycholic acid-induced rise in SCC was Ca^{2+}-dependent as it was reduced by removal of serosal Ca^{2+} in both the ileum (control: 56.5 ± 4.5 μA cm^{-2}; Ca^{2+}-free: 17.9 ± 3.2 μA cm^{-2}, n = 7, $P < 0.001$) and mid-intestine (control: 40.3 ± 3.4 μA cm^{-2}; Ca^{2+}-free: 2.5 ± 2.4 μA cm^{-2}, n = 7, $P < 0.001$).

Tetrodotoxin (10^{-5} M) did not affect the secretory response to ursodeoxycholic acid in either the ileum (n = 8, $P > 0.05$) or the mid-intestine (n = 8, $P > 0.05$), although the concentration used did inhibit the secretory response in intact sheets (ileum – control: 43.6 ± 7.0 μA cm^{-2}; tetrodotoxin: 16.7 ± 2.4 μA cm^{-2}, n = 8, $P < 0.01$; mid-intestine – control: 35.6 ± 4.3 μA cm^{-2}; tetrodotoxin: -2.3 ± 3.5 μA cm^{-2}, n = 7, $P < 0.001$). The first phase of the ileal response in intact sheets was, however, unaffected by the neurotoxin (control: 15.6 ± 1.7 μA cm^{-2}; tetrodotoxin: 13.0 ± 1.9 μA cm^{-2}, n = 8, $P > 0.05$).

Atropine (10^{-5} M) also failed to inhibit the secretory response to ursodeoxycholic acid in either the ileum (n = 6, $P > 0.05$) or mid-intestine (n = 6, $P > 0.05$), although the same concentration reduced the response to 10^{-3} M acetylcholine by 85.4 ± 3.6% (n = 7, $P < 0.001$).

The initial phase of the ileal response in stripped sheets was unaffected by any of these conditions ($P > 0.05$ in all cases). In addition, none of the conditions inhibited the rise in SCC induced by glucose ($P > 0.05$ in all cases, n = 4–7).

Involvement of mast cells in ursodeoxycholic acid action

The possible involvement of mast cells was tested by examining the effects of mast cell stabilizing agents and antagonists of mast cell degranulation products. Doxantrazole, which stabilizes both mucosal and connective tissue-type mast cells (Pearce et al 1982), caused a significant inhibition of the secretory response to ursodeoxycholic acid in both the ileum and mid-intestine, whereas sodium cromoglicate, which acts only on connective tissue-type mast cells (Pearce et al 1982), did not (Figure 4). Histamine and serotonin may be involved in the response to ursodeoxycholic acid as both the H_1 antagonist mepyramine and desensitization to 5-HT inhibited the rise in SCC (Figure 4). However, prostanoids

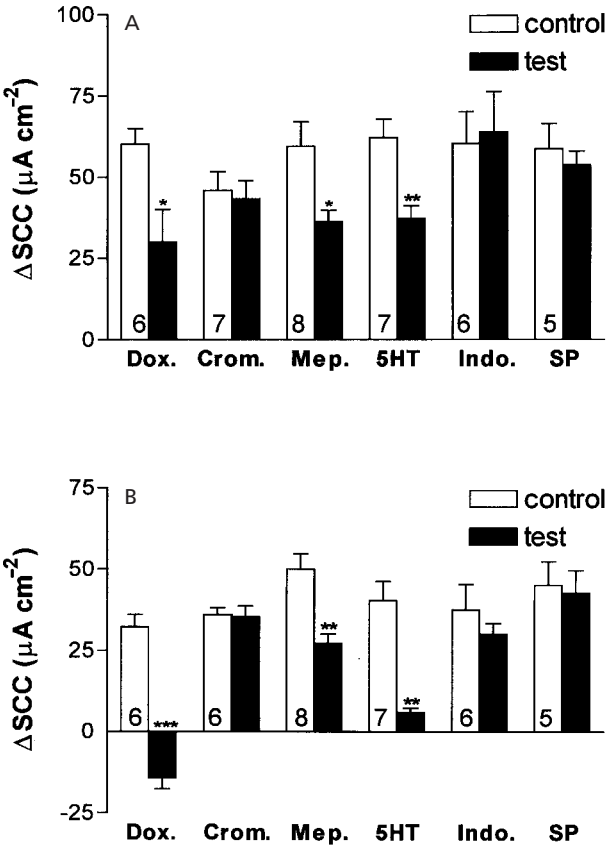

Figure 4 Involvement of mast cells in the secretory response of stripped sheets of mouse ileum (A) and mid-intestine (B) to ursodeoxycholic acid. The effects of mucosal + serosal doxantrazole (Dox., 10^{-3} M), mucosal + serosal sodium cromoglicate (Crom., 10^{-3} M), serosal indometacin (Indo., 5×10^{-5} M), serosal mepyramine (Mep., 10^{-4} M), desensitization to 5-HT (5HT, previous exposure to 10^{-4} M serosal 5-HT) or substance P (SP, previous exposure to 10^{-6} M serosal substance P) on the SCC response to 1 mM mucosal ursodeoxycholic acid are shown. Control sheets received an equivalent volume of vehicle. Each bar represents the mean ± 1 s.e.m. of the number of tissue pairs indicated in the columns and a paired *t*-test was used to assess significance: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

and substance P did not appear to make a contribution as neither indometacin nor desensitization to substance P had any effect (Figure 4). None of these treatments reduced the response to glucose ($P > 0.05$ in all cases, n = 4–6).

Effects of ursodeoxycholic acid on fluid transport

Under control conditions, mouse intestinal sacs exhibited a net uptake of fluid that represents a balance between absorptive and secretory processes (mid-intestine: 0.97 ± 0.08(n = 8) mL per g iww per 30 min; ileum: 0.49 ± 0.12(n = 8) mL per g iww per 30 min). In the mid-intestine mucosal ursodeoxycholic acid (1 mM) caused a significant inhibition of net fluid uptake ($P < 0.001$) and this effect was abolished by 10^{-3} M furosemide (ursodeoxycholic acid-induced change in fluid uptake – control: -0.52 ± 0.09(n = 8) mL per g iww per 30 min; + furosemide: -0.06 ± 0.05(n = 4) mL per g iww per 30 min, $P < 0.01$). This is consistent with the stimulation of secretion suggested by data from the intestinal sheet experiments. In the ileum, although fluid uptake was lower in the presence of mucosal ursodeoxycholic acid (0.30 ± 0.08(n = 8) mL per g iww per 30 min), this effect was not significant ($P > 0.05$). Active uptake of ursodeoxycholic acid in the ileum may offset its secretory effects, explaining the failure to detect any significant change in net fluid uptake when the bile acid was added to the mucosal solution. However, furosemide (10^{-3} M) restored fluid uptake in the presence of ursodeoxycholic acid to control levels (0.49 ± 0.02(n = 5) mL per g iww per 30 min), suggesting a secretory component to bile acid action in the ileum.

Ursodeoxycholic acid action in intestine from cystic fibrosis mice

Basal PD, SCC and resistance values in stripped sheets of both ileum and mid-intestine from wild-type mice did not differ significantly from those in tissues from Swiss MF1 mice ($P > 0.05$ in all cases). In ilea from CF mice the basal PD, SCC and resistance values did not differ significantly from those in wild-type mice. In the mid-intestine the PD and SCC were higher in CF tissues (CF: 1.0 ± 0.2 mV and 48.9 ± 9.6 μA cm^{-2}, n = 14; wild-type: 0.2 ± 0.2 mV and 5.5 ± 7.2 μA cm^{-2}, n = 4, $P < 0.05$ in both cases) and the resistance was lower (CF: 21.9 ± 1.4 Ω cm^2, n = 14; wild-type: 28.0 ± 1.1 Ω cm^2, n = 4, $P < 0.05$). The phenotype of the CF tissues was confirmed by testing the effects of acetylcholine and glucose. Secretory stimulation by 10^{-3} M acetylcholine induced a large increase in SCC in wild-type tissues, but this response was absent in CF tissues (wild-type: 182.5 ± 10.5 μA cm^{-2}, n = 4; CF: 4.3 ± 2.1 μA cm^{-2}, n = 14, $P < 0.001$). Addition of 10 mM glucose to the mucosal fluid caused similar increases in SCC in both types of tissue (wild-type: 114.2 ± 21.5 μA cm^{-2}, n = 4; CF: 95.2 ± 11.5 μA cm^{-2}, n = 13, $P > 0.05$).

In wild-type ileum mucosal ursodeoxycholic acid (1 mM) caused a biphasic increase in PDSCC similar to that observed in tissues from Swiss MF1 mice, but in CF

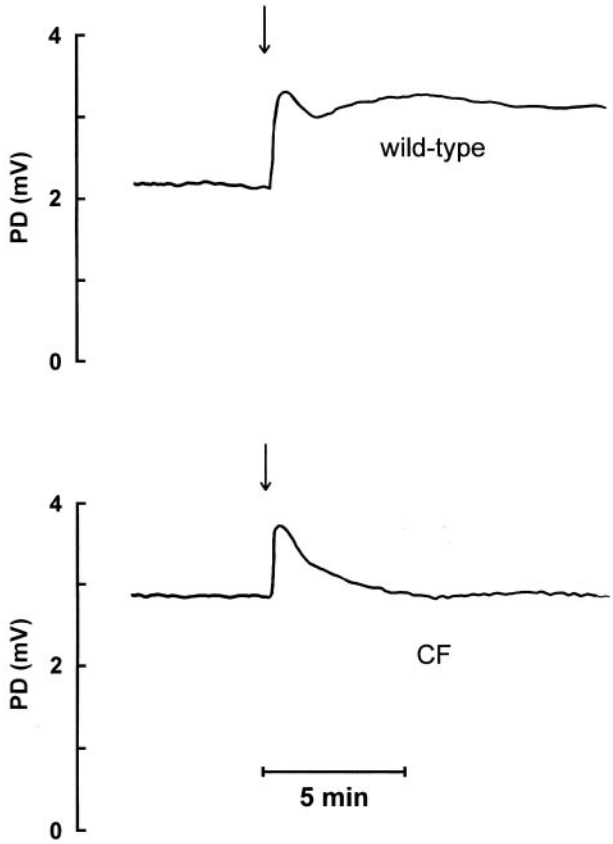

Figure 5 Typical response of stripped sheets of ileum from CF and wild-type mice to mucosal ursodeoxycholic acid. The PD is displayed as a function of time and 1 mM ursodeoxycholic acid was added to the mucosal solution at the point indicated by the arrow.

tissues only the initial phase was present (Figure 5). The magnitude of the initial phase in CF tissues ($28.9 \pm 3.6\ \mu A\ cm^{-2}$, n = 13) did not differ significantly ($P > 0.05$) from values obtained in ilea from wild-type ($29.7 \pm 3.6\ \mu A\ cm^{-2}$, n = 4) or Swiss MF1 mice ($31.6 \pm 1.1\ \mu A\ cm^{-2}$, n = 92). This residual response in CF tissues appears to represent bile acid absorption as it was inhibited by low mucosal Na^+ (control: $34.4 \pm 7.0\ \mu A\ cm^{-2}$; low Na^+: $8.7 \pm 4.9\ \mu A\ cm^{-2}$, n = 5, $P < 0.05$), but unaffected by 10^{-3} M furosemide (control: $30.0 \pm 2.8\ \mu A\ cm^{-2}$; furosemide: $23.9 \pm 4.2\ \mu A\ cm^{-2}$, n = 4, $P > 0.05$). The second phase of the ileal response to ursodeoxycholic acid was similar in tissues from wild-type ($43.2 \pm 4.7\ \mu A\ cm^{-2}$, n = 4) and Swiss MF1 ($55.2 \pm 1.8\ \mu A\ cm^{-2}$, n = 92) mice, but it was absent in CF tissues ($-5.2 \pm 3.3\ \mu A\ cm^{-2}$, n = 13, $P < 0.001$ compared with wild-type). Serosal application of ursodeoxycholic acid (1 mM) caused a monophasic increase in SCC in wild-type ilea ($25.2 \pm 4.8\ \mu A\ cm^{-2}$, n = 4), but had little effect in CF tissues ($5.1 \pm 1.9\ \mu A\ cm^{-2}$, n = 4, $P < 0.05$).

In wild-type mid-intestine mucosal ursodeoxycholic acid increased the SCC by $20.6 \pm 4.2\ \mu A\ cm^{-2}$ (n = 4), but this response was absent in CF tissues (-9.5 ± 2.3, n = 9, $P < 0.001$). Serosal application of ursodeoxycholic acid (1 mM) increased the SCC in wild-type tissues ($18.3 \pm 1.4\ \mu A\ cm^{-2}$, n = 4), but had little effect in CF tissues ($6.0 \pm 3.4\ \mu A\ cm^{-2}$, n = 9, $P < 0.05$).

Discussion

Ursodeoxycholic acid, when applied to either the mucosal or serosal side of the mouse tissue preparation, caused a secretory response in all regions of the normal small intestine. This was evident both from the Cl^--dependent rise in SCC generated by intestinal sheets and the furosemide-sensitive reduction in fluid uptake observed in intestinal sacs. Other bile acids were also capable of generating a secretory response, although there was some variability in their actions. Except for taurocholic acid, all the bile acids tested stimulated secretion in each of the intestinal regions, although the effective site of application varied, with no consistent pattern being observed. Taurocholic acid had no effect in the mid-intestine, regardless of its site of application, but it produced large responses when added mucosally to the ileum or serosally to the jejunum.

The secretory actions of ursodeoxycholic acid did not involve a neural pathway as tetrodotoxin did not inhibit its effects in stripped preparations. In this respect it differs from taurocholic acid whose secretory response in stripped ileum is inhibited by the neurotoxin (Hardcastle et al 2001). Tetrodotoxin did, however, reduce ursodeoxycholic acid-induced secretion in intact ileum and mid-intestine, suggesting that in this preparation at least part of the response is mediated by a neural pathway involving the myenteric plexus. Such a mechanism has also been implicated for other bile acids in rat small intestine both in-vivo (Karlström 1986) and in-vitro (Levin & Ayton 1995).

Even though a neural pathway does not contribute to ursodeoxycholic acid-induced secretion in stripped sheets, at least part of the response is mediated by an indirect action of the bile acid. There is evidence that ursodeoxycholic acid may act on mucosal mast cells as doxantrazole, but not sodium cromoglicate, inhibited its secretory actions. Mast cells have also been implicated in the secretory response of rat small intestine to antigen challenge (Crowe et al 1990) and a blunted response to bile acids has been observed in colonic

tissues from mast-cell-deficient mice (Gelbmann et al 1995). Antagonism of the actions of mast cell degranulation products was tested to identify the mediators contributing to ursodeoxycholic acid action. Histamine induces intestinal secretion via H_1 receptors (Hardcastle & Hardcastle 1987) and the H_1 antagonist mepyramine inhibited the secretory response to ursodeoxycholic acid. This is consistent with the finding that bile acids induce histamine release from a murine mast cell line that is functionally similar to mucosal mast cells (Quist et al 1991). Another mast cell mediator is serotonin, but because its secretory actions involve several different receptor subtypes and sites of action (Hardcastle & Hardcastle 1997) no selective inhibitor can abolish its effects. Nevertheless, the desensitization that follows 5-HT challenge in-vitro (Hardcastle et al 1994) can be used to test the involvement of this mediator in ursodeoxycholic acid action. Previous exposure to 5-HT reduced the secretory response to the bile acid in both the ileum and mid-intestine, suggesting that 5-HT does contribute to this effect. In contrast, there was no evidence for the involvement of prostanoids or substance P.

Bile acids can also induce secretion via a rise in cytosolic Ca^{2+} in a colonic cell line (Dharmsathaphorn et al 1989), indicating that they are capable of a direct action on transporting cells. The secretory response to ursodeoxycholic acid in mouse small intestine was inhibited in the absence of serosal Ca^{2+} and this may reflect a direct Ca^{2+}-dependent action on the enterocytes. However, the Ca^{2+} signalling pathway is also activated by histamine and serotonin (Barrett & Keely 2000). Moreover, Ca^{2+} is important for the release of mast cell degranulation products (Metcalfe et al 1997).

The secretory response in the mid-intestine and jejunum reached its maximum SCC more rapidly when ursodeoxycholic acid was added to the serosal compartment. This supports the concept that the bile acid is acting subepithelially to induce its effects and is consistent with an action on mucosal mast cells. Such an indirect mechanism of action has been suggested recently in studies of deoxycholic acid-induced secretion in rabbit and rat distal colon (Mauricio et al 2000) where secretion was observed in intact mucosa, but not in isolated crypts. In the ileum the secretory response to ursodeoxycholic acid was more rapid on mucosal application because there is an active mechanism for the reabsorption of bile acids in this region of the intestine (Hofmann 1998), that will accelerate the transport of ursodeoxycholic acid to its site of action.

In the ileum, ursodeoxycholic acid, as well as evoking a secretory response, also induced an initial increase in SCC that was unaffected by inhibitors of Cl^- secretion. It was, however, reduced when the mucosal Na^+ concentration was lowered, consistent with active Na^+-dependent bile acid absorption. This process is confined to the terminal ileum (Hofmann 1998) and in this study it was the only region of the small intestine to exhibit an initial Na^+-dependent increase in SCC. Ursodeoxycholic acid absorption has been shown to be Na^+-dependent in rat ileum, but not in the jejunum (Takikawa et al 1997), indicating that this bile acid can also be transported by the active reabsorptive process.

Intestinal sheets from transgenic CF mice exhibited the intestinal secretory defect observed in human patients with the disease (Taylor et al 1988) as they failed to respond to acetylcholine with an increase in SCC. The secretory response to mucosal and serosal ursodeoxycholic acid was also impaired in both the ileum and mid-intestine, but in the ileum the initial rise in SCC was still present. Although the magnitude of this response was similar to that observed in ilea from both wild-type and Swiss MF1 mice, its ionic basis may differ. In CF ilea only Na^+-dependent absorption occurs, while in normal ilea a secretory component may also be present. Consequently, lowering the mucosal Na^+ concentration to 25 mM caused a greater inhibition of the initial response in CF ilea than in normal tissues (CF: $80.3 \pm 17.4\%$, $n = 5$; Swiss MF1: $33.1 \pm 7.8\%$, $n = 6$, $P < 0.05$). Thus the Na^+-dependent component of the initial response was larger in CF tissues ($25.8 \pm 6.8\ \mu A\ cm^{-2}$, $n = 5$) than in ilea from Swiss MF1 mice ($8.7 \pm 1.9\ \mu A\ cm^{-2}$, $n = 6$, $P < 0.05$). This suggests that absorption of ursodeoxycholic acid, like that of sugars and amino acids (Baxter et al 1990), may be enhanced in CF. Previous studies of bile acid absorption in CF have produced conflicting data, with both normal absorption (de Rooij et al 1985; Thompson & Davidson 1988) and malabsorption (Fondacaro et al 1982; O'Brien et al 1983) being reported in human ileum. The availability of transgenic CF mice will allow this to be investigated in more detail.

This study has demonstrated that ursodeoxycholic acid induces secretion throughout the normal murine small intestine and that this response involves mucosal mast cells and the mast cell mediators histamine and serotonin. In contrast, CF intestine does not exhibit a secretory response to ursodeoxycholic acid. In the ileum there is evidence for the Na^+-dependent absorption of the bile acid and this may be enhanced in CF. The failure of secretion, together with enhanced absorption, will lead to excessive dehydration of the luminal contents in CF intestine and this may contribute to intestinal symptoms such as obstruction (Eggermont 1985).

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