Absorption of taurocholic acid by the ileum of normal and transgenic ΔF508 cystic fibrosis mice

J. Hardcastle, M. D. Harwood and C. J. Taylor

Abstract

Changes in intestinal transport in cystic fibrosis (CF) include both defective Cl^- secretion and alterations in absorption. This study focused on the effects of CF on the active re-absorption of bile acids in the ileum of normal and transgenic CF mice. Taurocholic acid absorption was monitored as changes in short-circuit current (SCC) in intact and stripped ileal sheets from normal (Swiss) and transgenic CF ($Cftr^{tm2Cam}$) mice with the ΔF508 mutation. Taurocholic acid uptake was measured directly in everted ileal sacs and in brush-border membrane vesicles (BBMVs) using radiolabelled bile acid. Taurocholic acid caused a biphasic increase in SCC in both intact and stripped ileal sheets from Swiss mice. The initial phase of the response was associated with active bile acid absorption as it was inhibited by a low mucosal Na^+ concentration, but unaffected by Cl^--free conditions, serosal furosemide or mucosal diphenylamine-2-carboxylic acid (DPC). The first phase was concentration-dependent and was reduced in the presence of other actively transported bile acids. Intact ileal sheets from wild-type $Cftr^{tm2Cam}$ mice also exhibited a biphasic SCC response to taurocholic acid, but in CF tissues the initial phase was reduced and the second phase was absent. Taurocholic acid was actively taken up by everted ileal sacs from Swiss mice. This process was inhibited by a low mucosal Na^+ concentration or the presence of other actively transported bile acids. A similar taurocholic acid uptake was observed in ileal sacs from wild-type mice, but in those from CF mice transport of the bile acid was significantly reduced. However, taurocholic acid uptake was similar in BBMVs from wild-type and CF ilea. Active absorption of taurocholic acid occurs in mouse ileum and this process is reduced in transgenic mouse models of CF with the ΔF508 mutation. However, this difference cannot be detected in an isolated preparation of brush-border membranes.

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Introduction

Bile acids are secreted into the intestinal lumen where they contribute to the processing of dietary fat. As they are not consumed by this process bile acids are actively reabsorbed by a Na^+-dependent mechanism localised to the enterocytes in the ileum and then returned via the portal circulation to the liver for re-secretion into the bile (Binder 1980). This enterohepatic circulation of bile acids is extremely efficient, with less than 10% escaping re-absorption and being lost in the faeces each day (Hofmann 1998). The first step in the uptake of bile acids involves a Na^+ gradient-driven co-transporter, the ileal bile acid transporter (IBAT), located on the luminal membranes of ileal enterocytes (Hofmann 1998). This operates in a similar fashion to the co-transporters responsible for Na^+-linked nutrient absorption.

In cystic fibrosis (CF) intestinal transport is disturbed, with both a failure of Cl^- secretion (Taylor et al 1987, 1988; Berschneider et al 1988; O'Loughlin et al 1991) and enhanced Na^+ and Na^+-linked nutrient absorption (Grubb & Boucher 1997; Baxter et al 1990). CFTR (cystic fibrosis transmembrane conductance regulator), the protein product of the CF gene, functions as a cAMP-regulated Cl^- channel (Riordan 1993) and is an integral component of the process responsible for electrogenic Cl^- secretion (Barrett & Keely 2000). However, CFTR also influences the behaviour of other transport proteins (Greger et al 2001). The possibility that CFTR may affect the behaviour of the IBAT is suggested by reports of bile acid malabsorption in CF patients (Fondacaro et al 1982; O'Brien et al 1993). However, another study failed to

detect any difference in ileal bile acid uptake between control and CF subjects (Thompson & Davidson 1988). The development of transgenic mouse models of CF provides an opportunity to examine the effects of the disease on the ileal mechanism for bile acid re-absorption in more detail. In this study we have used a mouse model in which the ΔF508 mutation, the most common mutation in man (Veeze 1992), has been introduced into the CF gene (Colledge et al 1995) to investigate bile acid re-absorption by the ileum in CF.

Materials and Methods

Chemicals

The following drugs were used: acetylcholine chloride, bovine serum albumin (BSA), cellobiose, dimethyl sulfoxide (DMSO), furosemide (frusemide), glycocholic acid, 5-hydroxytryptamine creatinine sulfate (5-HT), taurocholic acid, ursodeoxycholic acid (Sigma Chemical Company Ltd, Poole, UK); diphenylamine-2-carboxylic acid (DPC, N-phenylanthranilic acid; Fluka Chemicals, Gillingham, UK); glucose (Fisons Scientific Equipment, Loughborough, UK); mannitol (May & Baker, Dagenham, UK). ^{3}H-taurocholic acid (specific activity 129.5 GBq mmol^{-1}) was obtained from Perkin Elmer Life Sciences Inc. (Boston, MA). 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 Ethics Committee approval. Male Swiss mice, 20–30 g, 12–13 weeks old, were obtained from the Sheffield Field Laboratories. The $Cftr^{tm2Cam}$ transgenic cystic fibrosis (CF) mouse model (Colledge et al 1995), in which the ΔF508 mutation had been introduced into the CFTR gene, was used. Transgenic mice were bred in the Sheffield Field Laboratories and those used in the study included mice homozygous for the ΔF508 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 mouse ileum taken from the region just proximal to the caecum. Both intact and stripped (outer muscle layers and myenteric plexus removed) tissues were used. 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 (in mM: Na$^+$ 143.4, K$^+$ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl$^-$ 125.7, HCO$_3^-$ 24.9, H$_2$PO$_4^-$ 1.2, SO$_4^{2-}$ 1.2) 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 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 basal readings, bile acid was added to the mucosal or serosal fluid and readings were continued for a further 10 min.

In Cl$^-$-free conditions, all Cl$^-$ in the mucosal and serosal solutions was replaced with gluconate. To test the Na$^+$-dependence of the taurocholic acid response, the Na$^+$ concentration of the mucosal solution was reduced to 25 mM by replacing all the NaCl in the Krebs with isotonic mannitol. 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 was added to 5 mL bathing solution. Taurocholic acid was dissolved in 154 mM NaCl, glycocholic acid and ursodeoxycholic acid were dissolved in ethanol and in each case 100 μL stock solution was added to 5 mL bathing solution. Preliminary experiments indicated that the vehicles had no effect on basal electrical activity ($P > 0.05$ in all cases).

The phenotype of the CF mice was confirmed by testing the intestinal response to secretagogue challenge. Intact ileal sheets were prepared and after 5 min basal readings acetylcholine (10^{-3} M) or 5-HT (10^{-4} M) was added to the serosal solution.

Measurement of fluid and taurocholic acid uptake by everted sacs

Fluid and taurocholic acid uptakes were measured in everted sacs of ileum taken from the region immediately proximal to the caecum. Each sac was approximately 5 cm long and one preparation was obtained from each mouse. Sacs were filled with 0.1–0.2 mL Krebs bicarbonate saline containing 10 mM glucose and incubated at 37 °C for 30 min in 15 mL Krebs bicarbonate saline containing 10 mM mannitol, 1 mM taurocholic acid and 92.5 kBq/100 mL ^{3}H-taurocholic acid. During the incubation sacs were shaken at 80 oscillations per min. At the end of the incubation each sac was washed with 154 mM NaCl and gently blotted. It was then weighed and fluid uptake determined as the increase in weight of the filled sac during incubation. The serosal fluid was collected and the empty sac was cut into 1-cm pieces. The tissue was deproteinised by the addition of 1.25 mL 10% sodium tungstate and 1.25 mL 2/3N H$_2$SO$_4$, homogenised (Ultra-Turrax T25 with S 25 N–10 6 dispersing tool) for 1–2 min and filtered. Samples (100 μL) of initial mucosal fluid, final mucosal

fluid, final serosal fluid and gut homogenate were added to 3 mL Emulsifier Safe (Packard, Meriden, USA) and counted in a Packard Tri-Carb 1600TR liquid scintillation counter (Packard, Meriden, USA). Taurocholic acid uptake was determined as the amount taken up into the gut and serosal fluid. Fluid and taurocholic acid uptakes were related to the initial wet weight (iww) of the empty sac. The T/M ratio, the concentration of taurocholic acid in the tissue water compared with its concentration in the mucosal fluid at the end of the incubation, was used to determine whether active uptake (T/M ratio > 1) of the bile acid had occurred. The tissue water content was 80% initial wet weight (determined as 100–dry weight $\times$ 100/ wet weight) plus the volume of fluid taken up by the sac during incubation.

In low Na^+ conditions the Na^+ concentration of the mucosal solution was reduced to 25 mM by replacing all the NaCl in the Krebs with isotonic mannitol. The effects of glycocholic acid and ursodeoxycholic acid were tested by adding them to the mucosal fluid at the concentration indicated. The ethanol vehicle used to dissolve the bile acids was present at a concentration of 0.67% (v/v) and this had no significant effect on either fluid or taurocholic acid uptake ($P > 0.05$ in both cases).

Measurement of taurocholic acid uptake by brush-border membrane vesicles

Brush-border membrane vesicles (BBMVs) were isolated from transgenic CF ($Cftr^{tm2Cam}$) mice using a magnesium precipitation technique as described in detail by Klaren et al (2000). The distal 8 cm of small intestine was removed, flushed with ice-cold saline (150 mM NaCl and 20 mM HEPES–Tris, pH 7.4), cut open lengthwise and the mucosa scraped off with a microscope slide. To ensure that sufficient BBMVs were prepared, mucosae from 2 or 3 mice were pooled. The mucosae were homogenized in a 30-mL tissue grinder with a Teflon pestle (Wheaton, Millville, USA) in 15 mL ice-cold buffer (50 mM mannitol and 20 mM HEPES–Tris, pH 7.4). $MgCl_2$ was added to give a final concentration of 10 mM and the homogenate was stirred gently for 20 min. It was then spun for 10 min in a cooled (to 4 °C) JA-20 rotor at 4725 g_{max} in a Beckman J2-MC centrifuge. The resultant supernatant was spun at 41 400 g_{max} for 30 min and the pellet re-suspended in 1 mL buffer (300 mM mannitol, 0.1 mM $MgSO_4$, 20 mM HEPES–Tris, pH 7.4) by 25 passages through a 23-gauge needle. A further 15 mL buffer was added, the suspension was spun again at 4725 g_{max} for 15 min and the resultant supernatant spun at 41 400 g_{max} for 30 min. The final pellet was re-suspended in approximately 330 μL uptake buffer (200 mM mannitol, 0.1 mM $MgSO_4$, 20 mM HEPES–Tris, pH 7.4) to give a final protein concentration of approximately 1 mg mL^{-1}.

The protein content of the BBMV preparation was determined with a Coomassie Brilliant Blue reagent kit (Bio-Rad, München, Germany) using BSA as a standard. Alkaline phosphatase (a marker enzyme for brush-border membranes) and Na^+,K^+-ATPase (a marker enzyme for basolateral membranes) activities were measured as described previously (Klaren et al 2000). A 9.2 $\pm$ 2.3-fold (n = 6) enrichment of alkaline phosphatase activity occurred in the BBMV preparation (compared with the homogenate). This was significantly greater ($P < 0.01$) than the change in Na^+,K^+-ATPase activity (2.6 $\pm$ 0.4 fold, n = 6).

Taurocholic acid uptake was measured in duplicate using a rapid filtration protocol. BBMVs were incubated at 20 °C in a medium containing 100 μM taurocholic acid, 843 kBq mL^{-1} ^{3}H-taurocholic acid, 100 mM Na-gluconate, 0.1 mM $MgSO_4$, 20 mM HEPES–Tris, pH 7.4, for periods of 10–900 s. Uptake was terminated by adding an 18-fold surplus of ice-cold stop buffer (165 mM KCl, 100 μM taurocholic acid, 10 mM Tris, pH 7.4) and filtering immediately over a -80 kPa vacuum using a cellulose nitrate membrane filter with a pore diameter of 0.45 μm (Whatman, Maidstone, UK). The filter was rapidly rinsed with 2 $\times$ 4 mL ice-cold stop buffer and transferred to a vial, then 4.5 mL Emulsifier Safe (Packard, Meriden, USA) was added and the filter allowed to dissolve. Vials were then counted in a Packard Tri-Carb 1600TR liquid scintillation counter (Packard, Meriden, USA). Bile acid uptake was expressed as nmol taurocholic acid per mg protein.

Non-specific binding of taurocholic acid to the BBMVs was assessed by determining the effect of increasing the osmolarity of the medium by adding 100, 200, 300 or 400 mM cellobiose to the extravesicular solution. Vesicles were incubated for 30 min and taurocholic acid uptake was plotted against the reciprocal of extravesicular osmolarity. At infinite osmolarity the intravesicular space is zero and hence all uptake represents non-specific binding to the membrane. This was calculated as the intercept on the ordinate of the regression line.

Expression of results

Results are expressed as mean values $\pm$ s.e.m. of the number of observations indicated. Student's t-test, paired or unpaired as appropriate, was used to assess significance. For multiple comparisons analysis of variance, with post-hoc analysis by Scheffé's method, was used.

Results

Effect of taurocholic acid on transintestinal electrical activity in Swiss mice

The basal PD and SCC generated by intact and stripped ileal sheets from Swiss mice were 1.9 $\pm$ 0.1 mV and 68.8 $\pm$ 5.8 μA cm^{-2} (n = 38) and 1.6 $\pm$ 0.1 mV and 50.1 $\pm$ 1.8 μA cm^{-2} (n = 73), respectively, the serosa being positive with respect to the mucosa. Tissue resistance values were 29.5 $\pm$ 1.2 ohm.cm^2 and 32.9 $\pm$ 0.6 ohm.cm^2. Addition of taurocholic acid to the mucosal solution caused a biphasic increase in PD and SCC (Figure 1A) with intact and stripped sheets producing similar responses. The
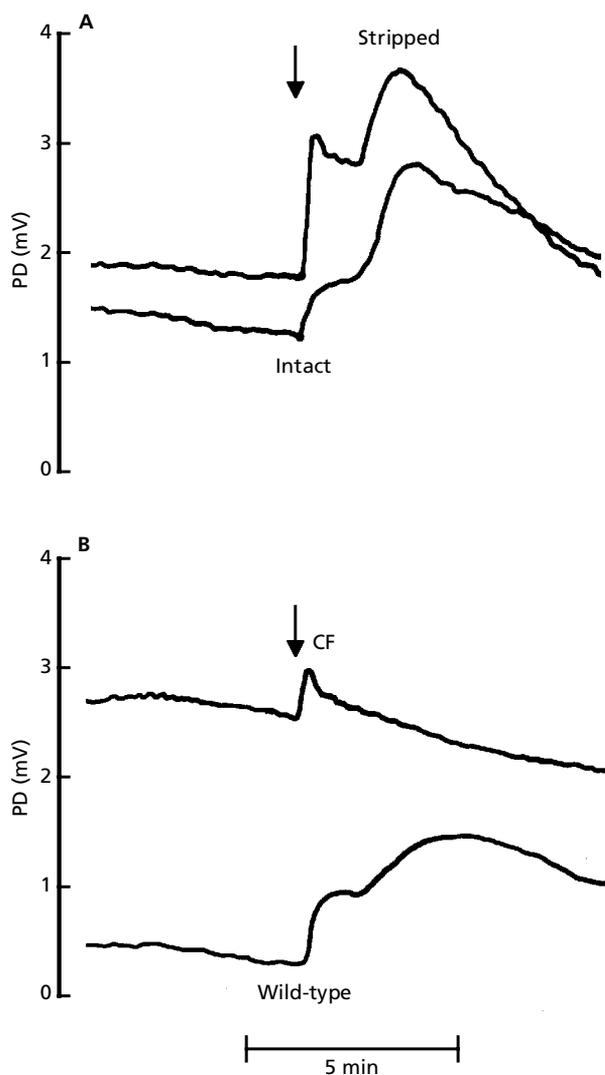
Figure 1 Typical changes in the potential difference (PD) across mouse ileum induced by mucosal application of taurocholic acid (2.5 mM) at the arrow. A. Comparison of taurocholic acid action in intact and stripped sheets from Swiss mice. B. Effect of taurocholic acid in intact ileum from wild-type and CF (*Cftr*^{tm2Cam}) mice.

first phase was $45.8 \pm 2.2\,\mu\mathrm{A\,cm}^{-2}$ in stripped sheets and $34.5 \pm 5.1\,\mu\mathrm{A\,cm}^{-2}$ in intact sheets, while the second phase was $97.0 \pm 10.4\,\mu\mathrm{A\,cm}^{-2}$ in stripped sheets and $101.8 \pm 17.4\,\mu\mathrm{A\,cm}^{-2}$ in intact sheets ($n = 7$, $P > 0.05$ in both cases). Serosal application of taurocholic acid (1 mM) to stripped sheets was without effect ($1.6 \pm 2.0\,\mu\mathrm{A\,cm}^{-2}$, $n = 4$). The initial phase of the response to mucosal bile acid was decreased when the Na^+ concentration of the mucosal solution was reduced to 25 mM, but it was unaffected by conditions that inhibit intestinal secretion (absence of Cl^-, serosal furosemide, mucosal DPC; Table 1). In contrast, the second phase was unaffected by low mucosal Na^+, but it was reduced by conditions that inhibit the secretory response (Table 1). This suggests that the initial phase of the electrical response to taurocholic acid is associated with its active Na^+-dependent absorption, while the second phase represents bile acid-induced secretion.

The magnitude of the absorptive phase of the SCC response was related to the taurocholic acid concentration in both intact and stripped sheets (Figure 2). A Lineweaver–Burke plot of the data provided apparent K_m and V_{max} values of 0.43 mM and $33.3\,\mu\mathrm{A\,cm}^{-2}$ in intact sheets and 0.50 mM and $50.0\,\mu\mathrm{A\,cm}^{-2}$ in stripped sheets. The presence of other actively transported bile acids in the mucosal solution inhibited the absorptive phase of the taurocholic acid-induced rise in SCC. In stripped ileal sheets, ursodeoxycholic acid (1 mM) reduced the response to taurocholic acid (1 mM) from 18.5 ± 3.3 to $-5.1 \pm 1.8\,\mu\mathrm{A\,cm}^{-2}$ ($n = 7$, $P < 0.001$), while glycocholic acid (1 mM) reduced the response from 29.9 ± 4.9 to $5.5 \pm 3.3\,\mu\mathrm{A\,cm}^{-2}$ ($n = 6$, $P < 0.01$).

Effect of taurocholic acid on transintestinal electrical activity in CF mice

The basal electrical activity was similar in intact ileal sheets from wild-type and CF mice, with PD, SCC and tissue resistance values of 1.6 ± 0.6 mV, $60.3 \pm 12.3\,\mu\mathrm{A\,cm}^{-2}$ and $23.8 \pm 3.6\,\mathrm{ohm.cm}^2$ ($n = 5$), respectively, in wild-type tissues and 1.4 ± 0.3 mV, $46.8 \pm 10.9\,\mu\mathrm{A\,cm}^{-2}$ and $30.0 \pm 0.3\,\mathrm{ohm.cm}^2$ ($n = 5$) in CF tissues ($P > 0.05$ in all cases). The phenotype of the tissues was confirmed

Table 1 Effect of taurocholic acid on stripped ileal sheets from Swiss mice.

Conditions	n	First peak ($\mu\mathrm{A\,cm}^{-2}$)		Second peak ($\mu\mathrm{A\,cm}^{-2}$)	
		Control	Test	Control	Test
Cl^--free	6	41.6 ± 4.1	32.3 ± 5.8	91.5 ± 13.3	44.7 ± 10.9**
Furosemide	5	41.8 ± 6.0	30.9 ± 8.9	79.5 ± 9.0	39.9 ± 12.5**
DPC	5	41.1 ± 4.8	22.9 ± 8.3	72.3 ± 13.9	19.2 ± 5.6*
Low $[Na^+]_m$	8	44.5 ± 8.1	17.7 ± 8.3*	97.2 ± 12.0	90.9 ± 12.1

Taurocholic acid (2.5 mM) was added to the mucosal solution and the first and second peaks of the SCC response recorded. The effects of Cl^--free conditions (Cl^- in mucosal and serosal solutions replaced with gluconate), furosemide (10^{-3} M in serosal solution, 25 μL DMSO in controls), DPC (10^{-3} M in mucosal solution, 25 μL ethanol in controls) and low mucosal Na^+ concentration ($[Na^+]_m = 25$ mM, NaCl replaced with mannitol) were determined. Values are mean $\pm$ s.e.m. of the number of tissue pairs (n) indicated. *$P < 0.05$; **$P < 0.01$ vs control (paired *t*-test).
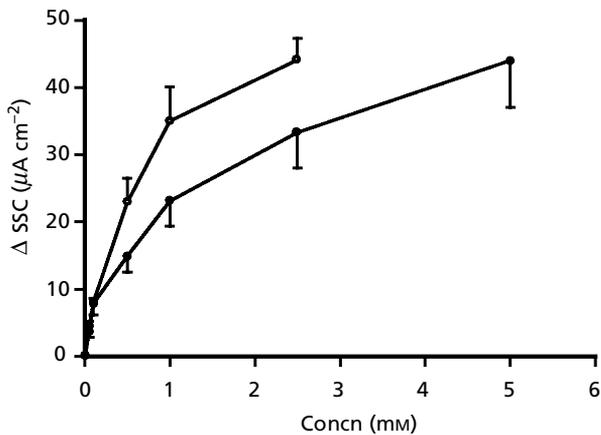
Figure 2 Relationship between the first phase of the rise in SCC and the taurocholic acid concentration. Taurocholic acid was added to the mucosal side of intact (●) and stripped (○) ileal sheets from Swiss mice. Each point represents the mean ± s.e.m. of 6–15 tissues.

Figure 3 Uptake of taurocholic acid by everted ileal sacs from Swiss mice. Sacs were incubated for 30 min in the presence of 1 mM taurocholic acid in the mucosal solution. In low Na^+ conditions the mucosal Na^+ concentration was reduced to 25 mM by replacement of NaCl with isotonic mannitol. The effects of 1 mM ursodeoxycholic acid (UDCA) or 1 mM glycocholic acid (GCA) in the mucosal solution were also tested. Each bar represents the mean ± s.e.m. of the number of sacs indicated. *$P < 0.05$ vs control (analysis of variance).

by testing their response to secretagogue challenge. Acetylcholine produced a very variable response in intact tissues from normal mice and in wild-type tissues 10^{-3} M acetylcholine increased the SCC by $25.0 \pm 14.3\,\mu A\,cm^{-2}$ (n = 5). Nevertheless this was significantly greater than the response observed in CF tissues ($-9.3 \pm 5.9\,\mu A\,cm^{-2}$ (n = 5, $P < 0.05$). The response to 5-HT was more consistent with 10^{-4} M 5-HT increasing the SCC by $75.2 \pm 20.2\,\mu A\,cm^{-2}$ (n = 5) in wild-type ilea and by $1.5 \pm 2.3\,\mu A\,cm^{-2}$ (n = 5, $P < 0.01$) in CF ilea. In wild-type tissues taurocholic acid (2.5 mM) caused a biphasic rise in SCC (Figure 1B) similar to that observed in tissues from Swiss mice, the magnitude of the first and second phases being 13.1 ± 1.7 and $32.0 \pm 6.2\,\mu A\,cm^{-2}$ (n = 5), respectively. In CF tissues the first phase of the response was still present (Figure 1B), although it was smaller ($2.0 \pm 3.3\,\mu A\,cm^{-2}$, n = 5, $P < 0.05$) than that obtained in wild-type tissues. The second phase was completely absent ($0.9 \pm 1.4\,\mu A\,cm^{-2}$, n = 5, $P < 0.01$).

Fluid and taurocholic acid uptake by everted ileal sacs

In everted ileal sacs from Swiss mice, fluid absorption was $0.58 \pm 0.07\,mL\,g^{-1}$ iww/30 min and taurocholic acid uptake was $5.29 \pm 0.75\,\mu mol\,g^{-1}$ iww/30 min (n = 14). The T/M ratio was 2.39 ± 0.24, indicating that taurocholic acid had been actively accumulated in the tissue. Taurocholic acid uptake was reduced when the mucosal Na^+ concentration was lowered (Figure 3). It was also inhibited in the presence of other actively absorbed bile acids (Figure 3). Fluid uptake was unaffected by any of these conditions ($P > 0.05$ in all cases). The effect of ursodeoxycholic acid was concentration dependent with 0.5, 1 and 2 mM reducing taurocholic acid uptake to 2.88 ± 0.23 (n = 5, $P > 0.05$), 2.31 ± 0.24 (n = 6, $P < 0.05$) and 1.15 ± 0.12 (n = 5, $P < 0.01$) $\mu mol\,g^{-1}$ iww/30 min, respectively.

In everted ileal sacs from wild-type mice, fluid absorption, taurocholic acid uptake and the T/M ratio were similar to values obtained in ilea from Swiss mice (Figure 4, $P > 0.05$ in all cases). In CF sacs however, taurocholic acid uptake and the T/M ratio were significantly reduced, although fluid uptake was similar (Figure 4).

Taurocholic acid uptake by BBMVs from wild-type and CF mice

In the presence of an inwardly-directed Na^+ gradient, taurocholic acid uptake by BBMVs from both wild-type and CF ilea exhibited an overshoot (Figure 5A). Peak uptake values were 4.90 ± 1.99 (n = 5) and 3.87 ± 0.83 (n = 5) nmol (mg protein)$^{-1}$ ($P > 0.05$) in vesicles from wild-type and CF ilea, respectively. The times taken to achieve peak uptake were 46 ± 7 and 37 ± 5 s ($P > 0.05$).

Taurocholic acid uptake exhibited significant non-specific binding, indicated by the effects of increasing the extravesicular osmolarity (Figure 5B). Extrapolating the regression line to conditions of infinite osmolarity demonstrated that a considerable uptake remained and this represented binding to the vesicle surface or incorporation into an osmotically-insensitive compartment rather than uptake into the intravesicular space. Similar observations have been reported for taurocholate uptake by human ileal BBMVs (Barnard & Ghishan 1987).

Discussion

This investigation has demonstrated the existence of a Na^+-dependent mechanism for the active re-absorption of bile acids by the terminal ileum of the mouse. In everted

A

B

C
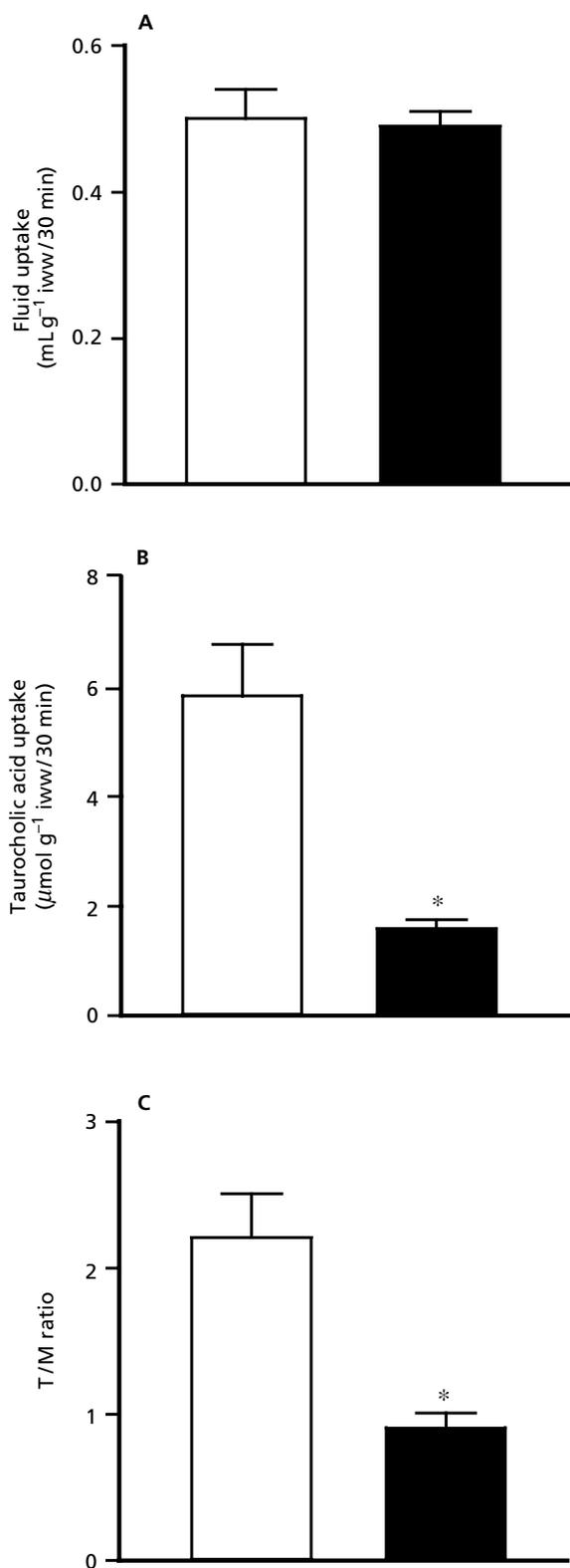
Figure 4 Comparison of fluid and taurocholic acid uptake by everted ileal sacs from wild-type (open bars) and CF (solid bars) mice (*Cftr*[tm2Cam]). A. Fluid uptake. B. Taurocholic acid uptake. C. T/M ratio. Each bar represents the mean ± s.e.m. of 8 sacs. *$P < 0.05$ vs control (unpaired *t*-test).

A

B
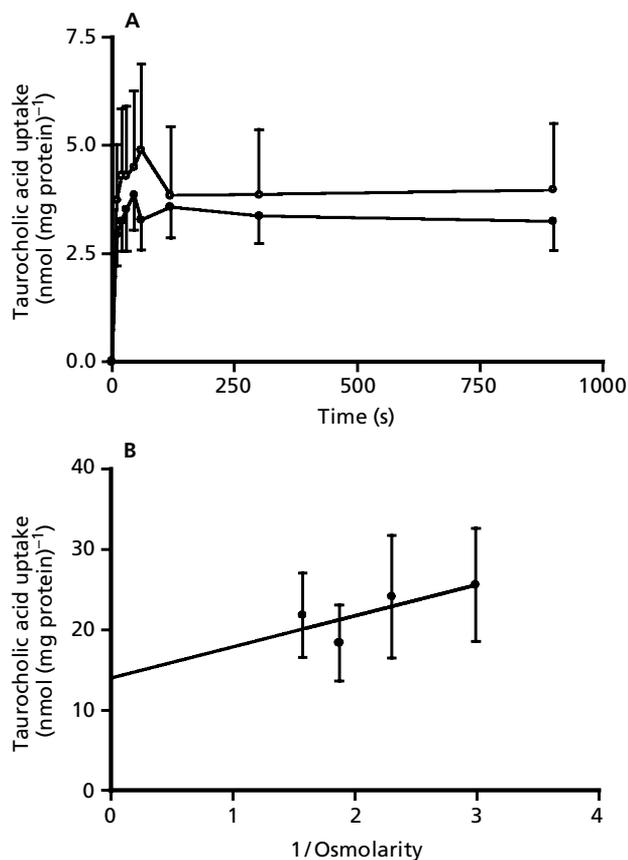
Figure 5 Taurocholic acid uptake by ileal brush-border membrane vesicles. A. Time course of taurocholic acid uptake by BBMVs from ilea of wild-type (○) and CF (●) mice (*Cftr*[tm2Cam]). The taurocholic acid concentration was 100 μM and uptake was measured in the presence of an inwardly-directed Na^+ gradient. Each point represents the mean ± s.e.m. of 5 preparations. B. Effect of increasing extravesicular osmotic pressure on the equilibrium uptake of taurocholic acid by BBMVs from ilea of Swiss mice. The taurocholic acid concentration was 100 μM, the cellobiose concentration was 100–400 mOsm L^{-1} and uptake was measured over 30 min. Each point represents the mean ± s.e.m. of 4 preparations and the regression line has been included.

sacs, taurocholic acid was accumulated within the tissue against its concentration gradient, leading to a T/M ratio significantly greater than unity and this process was inhibited when the Na^+ concentration of the mucosal solution was reduced. It was also inhibited by the presence of other actively transported bile acids that compete for the bile-acid binding site on the IBAT. The amount of taurocholic acid absorbed by mouse ileum is comparable with uptake of this bile acid by everted sacs of rat ileum (Caspary 1973) and by ileal biopsies from human patients (Ung et al 2002).

The activity of the IBAT could also be detected in both intact and stripped ileal sheets mounted in Ussing chambers. Mucosal application of taurocholic acid elicited a biphasic rise in SCC, as has been noted previously (Hardcastle et al 2001). The first phase of this response was reduced by a low mucosal Na^+ concentration, but was unaffected by

conditions that inhibit intestinal secretion. Thus removal of Cl^-, serosal furosemide (inhibits the Na^+, K^+, $2Cl^-$ co-transporter responsible for Cl^- accumulation within the enterocyte; Heintze et al 1983) or mucosal DPC (blocks luminal Cl^- channels; Sahi et al 1994) had no effect on the initial SCC response, although they did inhibit the second, secretory, phase. The magnitude of the initial SCC response was concentration dependent and the apparent K_m, in the order of 0.5 mM, was very similar to that (0.3 mM) obtained by direct measurement of taurocholic acid uptake by everted sacs of rat ileum (Caspary 1973).

The possibility that bile acid absorption was disturbed in CF was examined using a transgenic mouse with the same genetic defect found in the majority of CF patients. In wild-type mice, taurocholic acid uptake by everted ileal sacs was similar to that obtained in tissues from Swiss mice. In contrast, CF ilea exhibited a markedly reduced taurocholic acid uptake and a lower absorptive SCC response. The fact that the T/M ratio did not reach unity in CF sacs suggests that these tissues lack the capacity for active bile acid re-absorption. A similar conclusion was reached by Fondacaro et al (1982) in their study of tauro-cholic acid uptake by ileal biopsies from CF patients. Bile acid malabsorption has also been reported in a SeHCAT retention analysis of CF patients (O'Brien et al 1993), although an in-vivo perfusion study of human ileum failed to detect any difference in taurocholate and glycocholate uptake between control and CF subjects (Thompson & Davidson 1988). The only other study to use transgenic CF mice to investigate ileal bile acid re-absorption in CF found that, in contrast to the results presented here, tauro-cholic acid uptake was enhanced in an everted sleeve pre-paration of CF ileum (Stelzner et al 2001). However, this group used a knockout mouse in which no CFTR is produced, while we have used mice with the $\Delta F508$ muta-tion where aberrant CFTR is produced but, due to a trafficking defect, fails to reach its normal location at the luminal membrane of the enterocyte (Riordan 1993).

The reduction in taurocholic acid uptake observed in ilea from CF mice is in contrast to the enhanced absorption of sugars and amino acids reported in jejunal biopsies from CF patients (Baxter et al 1990), despite the fact that all the transport processes concerned are Na^+ dependent. The reason for this is not clear at present. It is possible that the effects of CFTR on the IBAT may differ from its effects on nutrient transporters. Alternatively, there may be species differences in the way in which CFTR interacts with Na^+-dependent transport systems.

The reduced taurocholic acid absorption observed in CF intestine was not mirrored in an isolated brush-border mem-brane preparation where taurocholic acid uptake was similar in wild-type and CF vesicles. A limited study of taurocholic acid uptake by ileal BBMVs from two CF patients also concluded that bile acid uptake was not abnormal in the disease (de Rooij et al 1985). Similarly, in human jejunum there is no difference in glucose uptake by BBMVs (Beesley et al 1996), although jejunal biopsies exhibit an enhanced glucose-dependent rise in SCC, an index of active Na^+-dependent glucose absorption (Baxter et al 1990). Both CFTR (O'Loughlin et al 1996; Ameen et al 2000) and the

IBAT (Stelzner et al 2000; St-Pierre et al 2001) are normally located at the luminal membrane of villous enterocytes, allowing possible interaction to occur. However, this may require the presence of intracellular components such as ATP and protein kinase A, that are required for CFTR activation (Riordan 1993), but are absent in the vesicle preparation.

This study indicates that bile acid malabsorption is evident in ileal tissues from CF mice. Severe malabsorp-tion can lead to decreased concentrations of bile acids in the intestinal lumen (Hofmann et al 1991) and this could exacerbate the maldigestion and malabsorption of fat that occurs in CF as a result of pancreatic exocrine insuffi-ciency (Dodge 1986).

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