Taurocholic acid-induced secretion in normal and cystic fibrosis mouse ileum

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Abstract

Bile acids cause secretion throughout the intestinal tract and this process contributes to maintaining the fluidity of intestinal contents. In cystic fibrosis (CF) defective intestinal secretion can lead to excessive dehydration of the luminal contents and the development of clinical symptoms. This study was designed to investigate bile acid-induced secretion in mouse ileum and to determine whether this process was defective in CF. Taurocholic acid-induced secretion was monitored as a rise in short-circuit current (SCC) in ileal sheets from normal (Swiss MF1) and transgenic CF mice. Taurocholic acid increased the SCC in both intact and stripped ileal sheets from Swiss MF1 mice. This effect was due to a stimulation of electrogenic Cl^- secretion as it was inhibited by Cl^--free conditions, serosal furosemide (frusemide), mucosal diphenyl-amine-2-carboxylic acid (DPC) and increased serosal K^+ concentration, without being affected by reduced mucosal Na^+ concentration. Taurocholic acid-induced secretion was inhibited by tetrodotoxin, indicating the involvement of a neural pathway, but this did not include capsaicin-sensitive afferent neurons or muscarinic cholinoreceptors. Mucosal mast cells also contributed to the response. Responses in tissues from transgenic wild-type mice were similar to those obtained with Swiss MF1 animals, but ilea from CF mice exhibited a lower basal SCC with significantly reduced secretory responses to acetylcholine and taurocholic acid. We concluded that taurocholic acid induces ileal secretion by a mechanism that entails activation of enteric nerves and degranulation of mucosal mast cells. Impaired bile acid-induced secretion in CF may contribute to luminal dehydration.

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Introduction

The secretory function of the intestine plays a vital role in maintaining the fluidity of the intestinal contents, facilitating the processes of digestion and absorption. Its importance is demonstrated in cystic fibrosis, a disease in which the secretory process fails throughout the intestinal tract (Taylor et al 1988; Hardcastle et al 1991), resulting in intestinal symptoms such as obstruction (Eggermont 1985). There are many endogenous agents that contribute to the secretory tone in healthy gut and these include the bile acids (Cooke 1994). Bile acid-induced secretion occurs in both the small intestine and colon (Binder 1980), but the ability of these agents to induce a secretory response in the cystic fibrosis intestine has not been tested. The development of transgenic cystic fibrosis mouse models has provided an opportunity to compare the transport activity of normal and cystic fibrosis intestine (Grubb & Gabriel 1997). There are, however, few reports on the effects of bile acids in normal mouse intestine, although these agents have been shown to induce secretion in the colon (Gelbmann et al 1995). This investigation was therefore

designed to characterize bile acid-induced secretion in the small intestine from normal mice and to determine whether this function is altered in cystic fibrosis. A preliminary report of the findings has been presented to the 13th Annual North American Cystic Fibrosis Conference (Hardcastle et al 1999).

Materials and Methods

Chemicals

The following drugs were used: acetylcholine chloride, atropine sulfate, capsaicin (8-methyl-N-vanillyl-6-nonenamide), sodium cromoglicate, dimethyl sulfoxide (DMSO), ethylene glycol-bis-(β-amino-ethyl) N,N'-tetra-acetic acid (EGTA), frusemide, 5-hydroxytryptamine creatinine sulphate (5-HT), indometacin, taurocholic acid, tetrodotoxin, verapamil (Sigma Chemical Co. Ltd, Poole, UK), diphenylamine-2-carboxylic acid (DPC, N-phenylanthranilic acid, Fluka Chemicals, Gillingham, UK), doxantrazole (3-(1H-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 carried out on male Swiss MF1 mice (age 12–13 weeks, body weight 20–30 g) obtained from the Sheffield Field Laboratories. A transgenic cystic fibrosis (CF) mouse model in which the $\triangle$F508 mutation has been introduced into the CFTR gene was also used: the *Cftr*^{tm1Eur} (Rotterdam) strain developed by Van Doorninck et al (1995). The transgenic mice were bred in the Sheffield Field Laboratories and animals used in the study included both wild-type mice and litter-mates homozygous for the $\triangle$F508 mutation. All mice were allowed free access to food and water and were killed by cervical dislocation in accordance with UK Home Office regulations.

Measurement of trans-intestinal electrical activity

The potential difference (PD), short-circuit current (SCC) and tissue resistance were measured across paired sheets of intact or stripped ileum taken from the region immediately adjacent to the caecum. The first (most distal) pair of sheets was used for the investigation of taurocholic acid action, with a second pair being used to establish the specificity of drug action. 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 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 taurocholic acid was added to the preparation and readings were continued for a further 10 min.

Normal Krebs bicarbonate saline contained (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. 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 serosal K$^+$ concentration was increased to 20 mM by replacing some NaCl with KCl; the mucosal Na$^+$ concentration was reduced to 25 mM by replacing all the NaCl with isotonic mannitol.

When inhibitors were used they were added to test sheets as soon as they were set up, with control sheets receiving an equivalent volume of vehicle. Vehicles used to prepare stock solutions were: capsaicin, 4% DMSO in saline for 10^{-6} M and 20% DMSO in saline for 5×10^{-6} M; frusemide, DMSO; DPC, ethanol; verapamil, methanol; and in each case 25 μL stock solution was added to 5 mL bathing solution. Indometacin was dissolved in 10% ethanol in 0.2% Na$_2$CO$_3$ and 100 μL was added to the serosal solution. None of the vehicles affected the response to taurocholic 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 and glucose (mucosal addition), and doxantrazole and sodium cromoglicate (mucosal + serosal addition). Taurocholic acid was generally added to the mucosal solution, but the effects of serosal application were also tested.

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 taurocholic acid 10 min later. Preliminary experiments indicated that this protocol completely eliminated a second response to 10^{-4} M 5-HT (control: 83.8 $\pm$ 9.7 μA cm^{-2}; test: -6.0 ± 4.5 μA cm^{-2}, n = 7, $P < 0.001$).

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 of stripped ileal sheets by 244.4 $\pm$ 9.6 μA cm^{-2} (n = 59).

In experiments on ileal sheets from CF mice, the genotype was confirmed by adding acetylcholine (10^{-3} M) to the serosal solution after 5 min of basal readings and continuing readings for a further 5 min. The serosal solution was then replaced and, after a 5-min recovery period, basal readings were taken for 5 min followed by the addition of 2.5 mM taurocholic acid to the mucosal solution and a further 10 min of readings. Preliminary experiments indicated that the prior application of acetylcholine had no significant effect on the subsequent response to taurocholic acid ($P > 0.05$).

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

Ileal sheets from Swiss MF1 mice generated a basal PD and SCC in which the serosal side of the tissue was positive with respect to the mucosal side. Basal PD, SCC and tissue resistance values were 1.9 $\pm$ 0.1 mV, 68.8 $\pm$ 5.8 μA cm^{-2} and 29.5 $\pm$ 1.2 ohm cm^2 (n = 38) in intact sheets and 1.6 $\pm$ 0.1 mV, 50.1 $\pm$ 1.8 μA cm^{-2} and 32.9 $\pm$ 0.6 ohm cm^2 (n = 73) in stripped sheets. Basal PD and SCC were very variable in intact sheets, which often exhibited spontaneous oscillations. This had been reported previously and can be eliminated by the addition of tetrodotoxin (Sheldon et al 1989). In this study it was found that removal of the outer muscle layers and myenteric plexus in the stripping procedure also eliminated spontaneous oscillations and produced a stable baseline.

Effect of taurocholic acid on trans-intestinal electrical activity

Addition of taurocholic acid to the mucosal solution caused a rapid rise in the ileal PD and SCC (Figure 1). There was an initial transient peak followed by a more gradual increase that reached a maximum at 2.3 $\pm$ 0.1 min. It was this second phase that we investigated. In stripped sheets 2.5 mM taurocholic acid increased the SCC by 106.8 $\pm$ 4.0 μA cm^{-2} and this coincided with a small decrease in tissue resistance of 2.5 $\pm$ 0.4 ohm cm^2 (n = 73). Tissue resistance continued to fall and 10 min after taurocholic acid application it had decreased by 5.3 $\pm$ 0.8 ohm cm^2, although the SCC remained elevated by 62.4 $\pm$ 4.0 μA cm^{-2} above the basal level. The effects of taurocholic acid were concentration dependent and were similar in intact and stripped sheets (Figure 2). In all subsequent studies with Swiss MF1 mice stripped sheets were used.

A second application of 2.5 mM taurocholic acid to the mucosal solution 10 min after the first did not elicit any increase in SCC (first application: 113.4 $\pm$ 16.0 μA cm^{-2}; second application: -15.4 ± 7.4 μA cm^{-2}, n = 6, $P < 0.001$), indicating that desensitization had occurred. However, if the initial application of the bile acid was washed out after 10 min, a second application made after a further 10 min could induce a response (first application: 120.9 $\pm$ 12.8 μA cm^{-2}; second application: 71.5 $\pm$ 12.8 μA cm^{-2}, n = 5, $P > 0.05$). Moreover, acetyl-
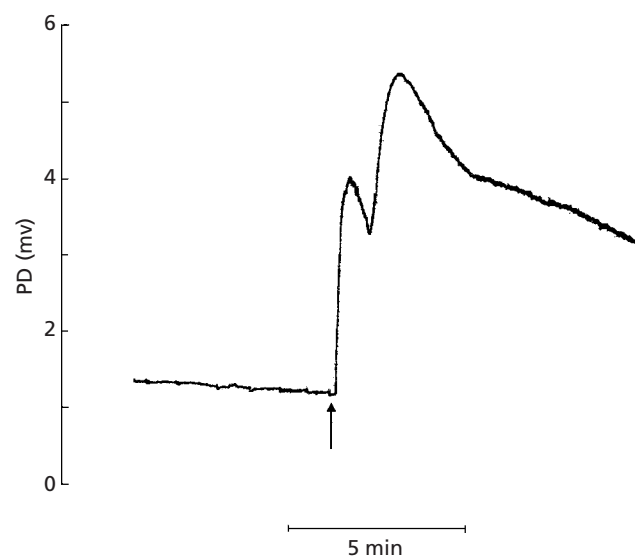

Figure 1 Typical response of a stripped sheet of mouse ileum to taurocholic acid. The potential difference (PD) is displayed as a function of time and 2.5 mM taurocholic acid was added to the mucosal solution at the point indicated by the arrow.
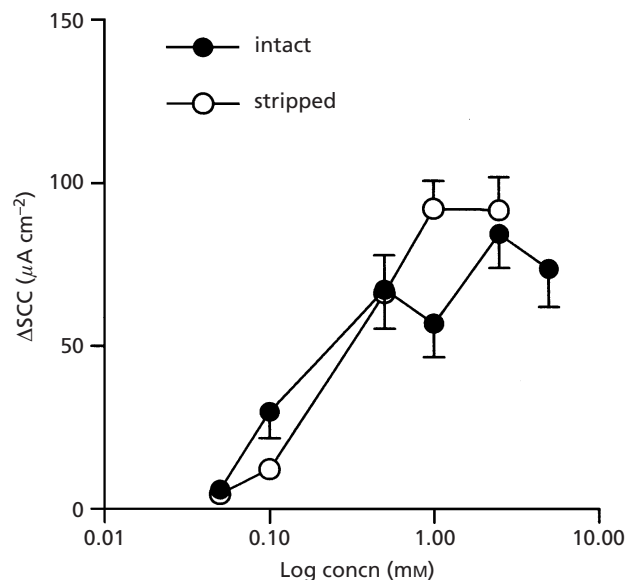
Figure 2 Concentration-dependence of taurocholic acid action in intact and stripped sheets of mouse ileum. Each point represents the mean ± 1 s.e.m. of 6–16 observations. SCC, short-circuit current.

Figure 3 Ionic basis of the electrical response of stripped sheets of mouse ileum to taurocholic acid. The effects of Cl^--free conditions ($-Cl^-$), 10^{-3} M serosal frusemide (Furo), 10^{-3} M mucosal diphenyl-amine-2-carboxylic acid (DPC), increasing the serosal K^+ concentration to 20 mM ($[K^+]_s$ 20 mM) or reducing the mucosal Na^+ concentration to 25 mM ($[Na^+]_m$ 25 mM) on the short-circuit current (SCC) response to 2.5 mM mucosal taurocholic acid are shown. Where appropriate, control sheets received an equivalent volume of vehicle. Each bar represents the mean ± 1 s.e.m. of the number of tissue pairs indicated and a paired t-test was used to assess significance: *$P < 0.05$; **$P < 0.01$.

choline added after taurocholic acid had been washed out caused an increase in SCC ($103.2 \pm 12.2 \,\mu$A cm^{-2}, n = 10) that did not differ significantly from that observed in control tissues ($136.3 \pm 18.4 \,\mu$A cm^{-2}, n = 10, $P > 0.05$). Thus the effects of taurocholic acid appear to be reversible.

Serosal application of taurocholic acid was much less effective, with 2.5 mM increasing the SCC by only $8.8 \pm 2.0 \,\mu$A cm^{-2}, compared with a response of $118.3 \pm 14.1 \,\mu$A cm^{-2} (n = 6, $P < 0.001$) to mucosal application in adjacent stripped sheets.

Ionic basis of the SCC response to taurocholic acid

The SCC response to 2.5 mM taurocholic acid was reduced by conditions that inhibit electrogenic Cl^- secretion (Figure 3). Cl^--free conditions reduced the response by $54.5 \pm 5.6\%$ (n = 6, $P < 0.001$) and serosal frusemide caused a $53.6 \pm 10.7\%$ (n = 5, $P < 0.01$) inhibition. Neither of these conditions affected the response to 10 mM glucose (n = 4, $P > 0.05$ in both cases). The Cl^- channel blocker DPC inhibited the effect of taurocholic acid by $67.4 \pm 12.9\%$ (n = 5, $P < 0.01$) at 10^{-3} M, but this concentration also reduced the rise in SCC induced by glucose, although the degree of inhibition ($32.7 \pm 7.1\%$, n = 6) was significantly less than that observed with taurocholic acid ($P < 0.05$). A re-
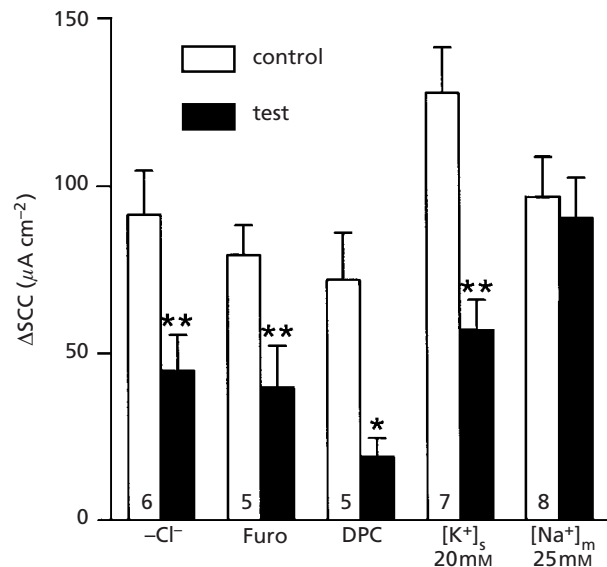
duced DPC concentration of 5×10^{-4} M still caused a significant inhibition of the taurocholic acid-induced rise in SCC (by $43.9 \pm 8.3\%$, n = 4, $P < 0.05$), but no longer affected the glucose response (n = 4, $P > 0.05$). Increasing the serosal K^+ concentration to 20 mM to limit the hyperpolarization due to opening of basolateral K^+ channels reduced the taurocholic acid response by $50.0 \pm 11.7\%$ (n = 7, $P < 0.01$) without affecting the response to glucose (n = 4, $P > 0.05$). In contrast, reducing the mucosal Na^+ concentration to 25 mM inhibited the glucose-dependent rise in SCC by $54.5 \pm 4.9\%$ (n = 8, $P < 0.001$), but did not affect the increased SCC induced by taurocholic acid ($P > 0.05$). It also failed to affect the rise in SCC induced by 10^{-3} M acetylcholine (control: $210.2 \pm 21.7 \,\mu$A cm^{-2}; low mucosal Na^+: $206.5 \pm 28.8 \,\mu$A cm^{-2}, n = 4, $P > 0.05$).

Ca^{2+}-dependence of taurocholic acid action

The rise in SCC induced by taurocholic acid was Ca^{2+}-dependent as it was reduced both by removal of serosal Ca^{2+} and by the addition of verapamil to the serosal
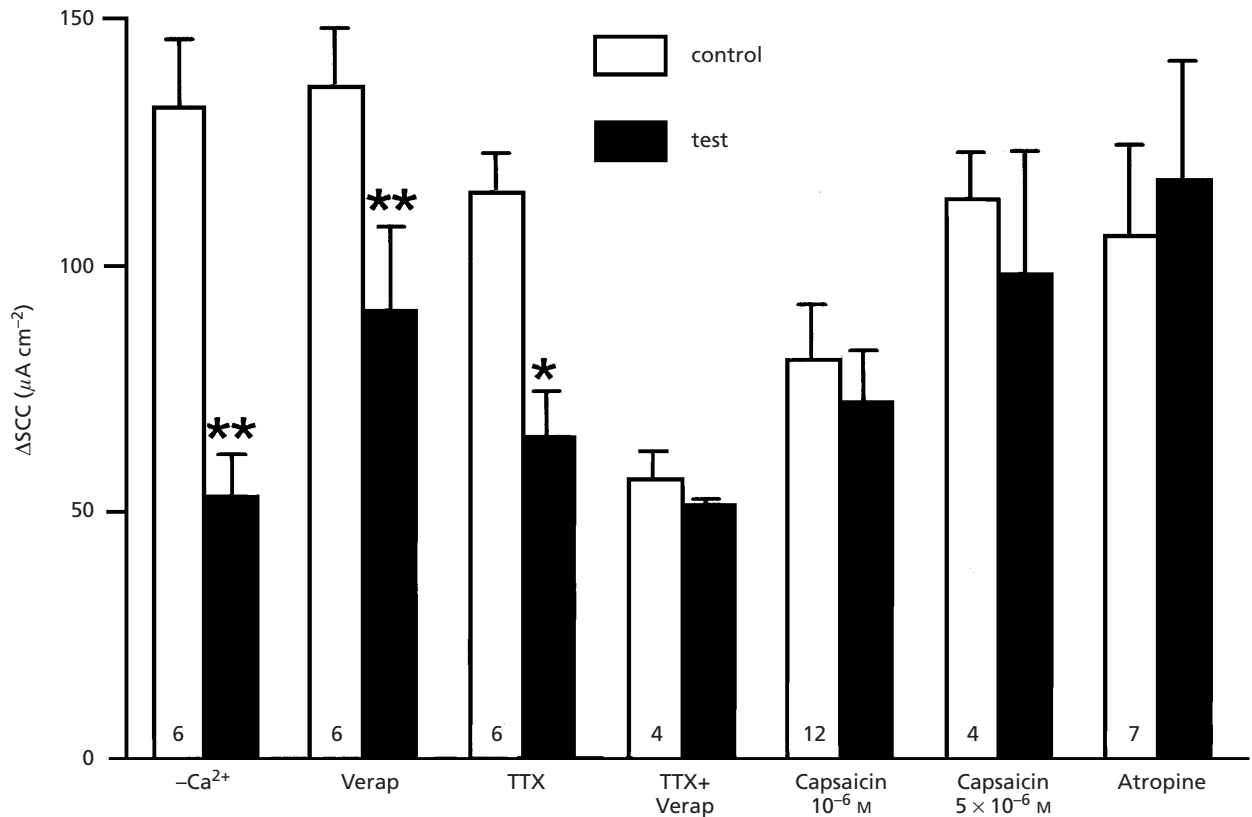
Figure 4 Involvement of Ca^{2+} and neural mechanisms in the response of stripped sheets of mouse ileum to taurocholic acid. The effects of the absence of serosal Ca^{2+} ($-Ca^{2+}$) or the presence of serosal verapamil (Verap, 10^{-4} M), tetrodotoxin (TTX, 10^{-5} M), capsaicin (10^{-6} M or 5×10^{-6} M) or atropine (10^{-5} M) on the SCC response to 2.5 mM mucosal taurocholic acid are shown. To test the effects of verapamil in the presence of tetrodotoxin (TTX + Verap), control sheets were exposed to serosal tetrodotoxin and test sheets to serosal tetrodotoxin and verapamil. 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 and a paired t-test was used to assess significance: $*P < 0.05$; $**P < 0.01$. scc, short-circuit current.

solution (Figure 4). It is not clear, however, whether this represented the involvement of Ca^{2+} at the level of the enterocyte. Neither removal of serosal Ca^{2+} (n = 5) nor the presence of verapamil (n = 4) affected the response to glucose ($P > 0.05$ in both cases).

Involvement of neural pathways in taurocholic acid action

Tetrodotoxin reduced the effect of taurocholic acid by $41.7 \pm 8.5\%$ (n = 6, $P < 0.01$), indicating that neural mechanisms contribute to the response (Figure 4). In the presence of the neurotoxin verapamil caused no further inhibition, suggesting that the Ca^{2+}-dependence of taurocholic acid action represented an effect on the neural pathways involved in the response.

There was no evidence for the involvement of capsaicin-sensitive afferent neurons as this agent did not

inhibit the rise in SCC induced by taurocholic acid. Atropine did not inhibit the taurocholic acid response, although it reduced the effect of 10^{-3} M acetylcholine by $85.4 \pm 3.6\%$ (n = 7, $P < 0.001$), thus muscarinic cholinoreceptors make no contribution (Figure 4).

None of the experimental conditions designed to investigate the involvement of neural pathways inhibited the rise in SCC induced by glucose ($P > 0.05$ in all cases, n = 4–7).

Involvement of mast cells in taurocholic 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. Dox-antrazole, which stabilizes both mucosal and connective tissue-type mast cells (Pearce et al 1982), caused a
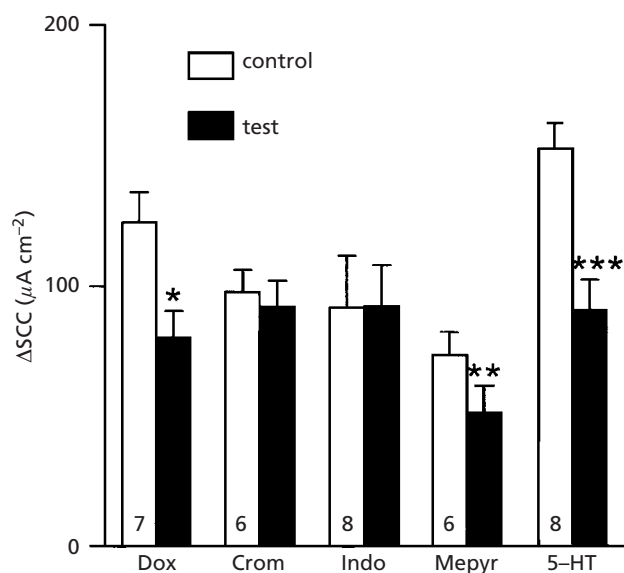
Figure 5 Involvement of mast cells in the response of stripped sheets of mouse ileum to taurocholic acid. The effects of mucosal + serosal doxantrazole (Dox, 10^{-3} M), mucosal + serosal sodium cromoglicate (Crom, 10^{-3} M), serosal indometacin (Indo, 5×10^{-5} M), serosal mepyramine (Mepyr, 10^{-4} M), or desensitization to 5-HT by previous exposure to 10^{-4} M serosal 5-HT on the short-circuit current (SCC) response to 2.5 mM mucosal taurocholic 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 and a paired *t*-test was used to assess significance: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 1 Basal electrical activity and SCC responses to serosal acetylcholine (10^{-3} M) and mucosal taurocholic acid (2.5 mM) in ileal sheets from transgenic CF mice (*$Cftr^{tm1Eur}$*).

	Wild-type (n = 10)	CF (n = 8)	*P*
PD (mV)	1.3 ± 0.3	1.0 ± 0.2	> 0.05
SCC (μA cm^{-2})	53.9 ± 9.4	29.5 ± 6.8	< 0.05
Resistance (ohm cm^2)	24.4 ± 1.6	35.9 ± 2.1	< 0.001
Acetylcholine (μA cm^{-2})	136.3 ± 18.4	11.3 ± 1.6	< 0.001
Taurocholic acid (μA cm^{-2})	89.2 ± 13.1	21.0 ± 2.9	< 0.001

Results are expressed as mean ± 1 s.e.m. of n animals and values from CF mice homozygous for the △F508 mutation were compared with those from wild-type controls, with an unpaired *t*-test used to assess the significance of any differences observed. PD, potential difference; SCC, short-circuit current.

significant inhibition of the taurocholic acid-induced rise in SCC, whereas sodium cromoglicate, which acts only on connective tissue-type mast cells (Pearce et al 1982), did not (Figure 5). Increased prostanoid production did not appear to contribute to taurocholic acid action as indometacin did not reduce the SCC response. However, both the H$_1$ antagonist mepyramine and desensitization to 5-HT caused an inhibition (34.4 ± 10.8%, n = 6, $P < 0.05$ and 41.4 ± 5.1%, n = 8, $P < 0.001$, respectively; Figure 5), suggesting that histamine and 5-HT are involved in the response to taurocholic acid. None of these treatments reduced the response to glucose ($P > 0.05$ in all cases, n = 4–6).

Taurocholic action in ilea from cystic fibrosis mice

Basal PD and SCC values in stripped ileal sheets from wild-type Rotterdam mice did not differ significantly from those in preparations from Swiss MF1 mice ($P > 0.05$ in both cases), although tissue resistance was slightly lower (Table 1). Acetylcholine caused a large increase in SCC and this was followed by a taurocholic acid response that was not significantly different from that in Swiss MF1 mice ($P > 0.05$). In ilea from CF mice homozygous for the △F508 mutation, the basal SCC was lower, and tissue resistance was higher. An increased tissue resistance in CF intestine has also been reported for human rectal biopsies (Mall et al 1999). The SCC responses to both acetylcholine and taurocholic acid were greatly reduced, consistent with impaired secretion in CF intestine (Table 1).

Discussion

Both intact and stripped sheets of mouse ileum responded to mucosal application of taurocholic acid with a concentration-dependent rise in SCC. This response was inhibited in the absence of Cl$^-$ by serosal frusemide, which blocks the Na$^+$,K$^+$,2Cl$^-$ co-transporter responsible for the accumulation of Cl$^-$ within the enterocyte (Heintze et al 1983), mucosal DPC, which blocks luminal Cl$^-$ channels (Sahi et al 1994) or the absence of Cl$^-$, consistent with a stimulation of electrogenic Cl$^-$ secretion. Increasing the serosal K$^+$ concentration to reduce the basolateral hyperpolarization that contributes to the maintenance of Cl$^-$ secretion (Hardcastle & Hardcastle 1987a) also inhibited the taurocholic acid-induced rise in SCC, further supporting the hypothesis that the electrical response reflected a secretion of Cl$^-$. The secretory response to taurocholic acid was observed at concentrations in the same range as those reported to be present in the intestinal lumen

(Northfield & McColl 1973), suggesting that it represents a normal aspect of gut function.

Bile acids are actively absorbed in the terminal ileum by a Na^+-dependent mechanism that could also generate electrical activity (Hofmann 1998). It does not, however, appear to contribute to the SCC response observed in this study, as reducing the mucosal Na^+ concentration to 25 mM had no effect, although it reduced the glucose-dependent rise in SCC, which is generated by a similar Na^+-dependent mechanism (Schultz & Zalusky 1964), by 55%.

Activation of the enteric nervous system was responsible for at least part of the taurocholic acid-induced secretion as tetrodotoxin inhibited the SCC response to the bile acid by 42%. However, muscarinic cholino-receptors were not involved. Neural mechanisms have also been shown to contribute to bile acid-induced secretion in rat small intestine, where the involvement of presynaptic cholinergic neurons and post-synaptic non-adrenergic, non-cholinergic neurons was demonstrated (Karlström 1986; Levin & Ayton 1995). In rat ileum it is the myenteric plexus that contributes to taurocholic acid-induced secretion as tetrodotoxin inhibits the response in intact tissues, but not in stripped preparations where the myenteric plexus is removed (Levin & Ayton 1995). In contrast, other components of the enteric nervous system must be involved in mouse ileum as tetrodotoxin was effective in stripped tissues. Activation of the enteric nervous system did not occur via stimulation of capsaicin-sensitive afferent neurons, as pre-treatment with capsaicin did not alter the response of mouse ileum to taurocholic acid. Similar observations have been made in the rat (Levin & Ayton 1995). It is possible that taurocholic acid could act on immune elements within the gut wall to release mediators that activate the enteric nerves, as an intimate relationship exists between the intestinal neural and immune systems (Cooke 1994). In mouse colon, histamine and mast cells have been implicated in the response to bile acids (Gelbmann et al 1995) and in this study the action of taurocholic acid was reduced by doxantrazole, a mast-cell stabilizing agent, suggesting that a similar mechanism operates in the ileum. The failure of sodium cromoglicate to cause any inhibition indicates that mucosal mast cells were involved, the type that has also been shown to mediate the intestinal secretory response to antigen challenge (Crowe et al 1990). Further evidence for the involvement of mast cells comes from the effects of antagonists of mast-cell degranulation products. Taurocholic acid action was inhibited by mepyramine, an antagonist at the H_1 receptors which are responsible for histamine-mediated intestinal secretion (Hardcastle & Hardcastle 1987b). Another mast-cell mediator is 5-HT, whose secretory actions involve a number of different 5-HT receptor subtypes and sites of action (Hardcastle & Hardcastle 1997), with the result that no selective antagonist is capable of abolishing the response. However, in-vitro desensitization occurs following 5-HT challenge (Hardcastle et al 1994) and this provided an experimental approach to test the involvement of 5-HT in taurocholic acid action. Taurocholic acid was found to be less effective in eliciting a secretory response in ileal sheets that had previously been de-sensitized by exposure to 5-HT, implicating 5-HT involvement in the effects of the bile acid. 5-HT has also been shown to contribute to bile salt-induced secretion by rat jejunum in-vivo (Timar Peregrin et al 1999). In contrast, there was no evidence from our investigation for the involvement of prostanoids.

It appears that a number of mediators contribute to taurocholic acid-induced secretion in mouse ileum. Their release may involve Ca^{2+}-dependent mechanisms as the SCC response to bile acid was inhibited by lack of Ca^{2+} or the presence of the Ca^{2+}-channel blocker verapamil. Since verapamil caused no further inhibition in the presence of tetrodotoxin it is likely that it is the release of neurotransmitters that is Ca^{2+} dependent rather than the action of the mediators on the transporting cells. In rat small intestine, bile-acid activation of the enteric nervous system occurs, at least in part, via the Ca^{2+}-dependent release of 5-HT from enterochromaffin cells (Timar Peregrin et al 1999). The results of this investigation are consistent with the operation of a similar mechanism of action in mouse ileum.

It is clear from this study that indirect mechanisms contribute to the secretory response of mouse ileum to taurocholic-acid stimulation. A similar conclusion has been reached with regard to deoxycholic acid in a recent report of its actions on ion transport in rabbit and rat distal colon (Mauricio et al 2000), where secretion was observed in intact mucosa, but not in isolated crypts. The possibility that bile acids might also act directly on the transporting cell cannot, however, be ruled out. They have been shown to activate ion channels associated with secretion in T84 cells (Devor & Frizzell 1993), a colonic cell line that exhibits many of the transport characteristics observed in normal enterocytes, suggesting that the transporting cells themselves possess the ability to respond to bile acid challenge.

These studies established the nature of the taurocholic acid response in mouse ileum and laid the foundations for the investigation of bile acid-induced secretion in intestinal tissue from transgenic CF mice. Ileal tissues from wild-type mice exhibited taurocholic-acid re-

sponses that were similar to those observed in Swiss MF1 mice, but there was a clear distinction between wild-type and CF mice. The tissues from the CF mice generated a lower basal SCC. This has also been reported for both human (Baxter et al 1990; Hardcastle et al 1991; Mall et al 1999) and mouse (Grubb & Gabriel 1997) intestine and can be attributed to the absence of basal Cl⁻ secretion. In stripped ileal preparations from CF mice a residual secretory response to acetylcholine was observed, similar to findings in other studies using transgenic CF mouse models (Grubb & Gabriel 1997). The response was, however, significantly smaller than that generated by wild-type tissues, indicating that the intestine from this CF mouse model exhibits the defective secretory response characteristic of the human disease. The secretory effects of taurocholic acid were also reduced, but not abolished, in CF tissues. Such residual secretory activity is not observed in ileal sheets from the *Cftr*^{tm2Cam} mouse model where neither acetylcholine nor taurocholic acid increased the SCC (Hardcastle et al 1999). Although these mice are also homozygous for the △F508 mutation, they have a more severe phenotype than the *Cftr*^{tm1Eur} mice used in this study and the fact that they do not exhibit any secretory activity suggests that the residual responses observed in this investigation do indeed represent Cl⁻ secretion. Thus it can be concluded that bile acid-induced secretion is abnormal in CF.

These studies have demonstrated that taurocholic acid can induce secretion in the ileum of normal mice and this response involves neural and immune elements within the gut wall. Moreover, in CF intestine, this response, like that to other secretagogues, is defective.

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