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Small intestinal glucose absorption in cystic fibrosis: a study in human and transgenic ΔF508 cystic fibrosis mouse tissues

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Abstract

Intestinal transport is disturbed in cystic fibrosis (CF), with both defective Cl^- secretion and changes in absorption being reported. We have examined the effects of the disease on Na^+-dependent glucose absorption by the small intestine. Active glucose absorption was monitored as changes in short-circuit current (SCC) in intact and stripped intestinal sheets from normal (Swiss) and transgenic CF (*Cftr*^{tm1Eur} and *Cftr*^{tm2Cam}) mice with the ΔF508 mutation, and in jejunal biopsies from children with CF and normal controls. Na^+-dependent glucose uptake at the luminal membrane was measured in brush-border membrane vesicles (BBMVs). Intact and stripped sheets of jejunum and mid-intestine from Swiss mice exhibited a concentration-dependent increase in SCC with glucose. Apparent K_m values were similar in the two preparations, but the apparent V_{max} was greater in stripped sheets. This difference was not due to a loss of neural activity in stripped sheets as tetrodotoxin did not influence the glucose-induced SCC in intact sheets. Similar results were observed in stripped sheets of jejunum and mid-intestine from wild-type *Cftr*^{tm1Eur} mice, but in tissues from CF mice the apparent V_{max} value was reduced significantly. A lower V_{max} was also obtained in intact sheets of mid-intestine from CF (*Cftr*^{tm2Cam}) mice. Jejunal biopsies from CF patients however, exhibited an enhanced glucose-dependent rise in SCC. Na^+-dependent uptake by BBMVs from CF (*Cftr*^{tm1Eur}) mice was not reduced compared with wild-type and Swiss BBMVs. It was concluded that, in contrast to human intestine, intestinal glucose absorption was reduced in transgenic mouse models of CF with the ΔF508 mutation, but that this could not be detected in an isolated preparation of brush-border membranes. Transgenic mouse models of CF may not accurately reflect all aspects of intestinal dysfunction in the human disease.

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Introduction

Cystic fibrosis transmembrane conductance regulator (CFTR), the protein product of the cystic fibrosis (CF) gene, not only acts as a cAMP-regulated Cl^- channel (Riordan 1993), but influences the behaviour of other transport proteins, e.g. the epithelial Na^+ channel (ENaC), the outwardly-rectifying Cl^- channel (ORCC) and aquaporins (see Greger et al 2001). CFTR is expressed throughout the intestinal tract (Strong et al 1994) where it is an integral component of the process responsible for the electrogenic secretion of Cl^- ions (Barrett & Keely 2000), the driving force for intestinal fluid secretion. In CF, where CFTR is abnormal, Cl^- secretion is defective (Taylor et al 1987, 1988; Berschneider et al 1988; O'Loughlin et al 1991) and the resulting failure of fluid secretion contributes to the intestinal symptoms of the disease.

The small intestine has both an absorptive and a secretory capacity, although its major role is the absorption of nutrients, electrolytes and water. It is possible that CFTR may affect absorptive processes in addition to its known effects on secretion. In jejunal biopsies from CF patients the Na^+-dependent absorption of sugars and amino acids is enhanced, a change that reflects an increase in the maximum capacity of the absorptive process (Baxter et al 1990; Hardcastle et al 1994). The development of transgenic CF mouse models has provided an opportunity to study the transport activity of CF intestine in more detail (Grubb & Gabriel 1997). In this investigation the Na^+-dependent absorption of glucose was examined in a CF mouse model in which

the ΔF508 mutation, found in 70–80% CF patients (Veeze 1992), had been introduced into the CFTR gene (Colledge et al 1995; Van Doorninck et al 1995). The findings were compared with the effects of CF on glucose absorption by human small intestine.

Materials and Methods

Chemicals

The following drugs were used: acetylcholine chloride, bovine serum albumin (BSA), tetrodotoxin (all three from Sigma Chemical Company Ltd, Poole, UK), L-alanine (Koch-Light Laboratories Ltd, Haverhill, UK), glucose (Fisons Scientific Equipment, Loughborough, UK), α-methyl-D-glucoside (Mann Research Laboratories, New York, NY), mannitol (May & Baker, Dagenham, UK), and phlorizin (Phase Separations Ltd, Queensferry, UK). D-[2-^{3}H]-Glucose (specific activity 518 GBq mmol^{-1}) was obtained from Amersham Biosciences (Amersham, 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 the approval of the local Ethics Committee. Male Swiss mice (age 12–13 weeks, body weight 20–30 g) were obtained from the Sheffield Field Laboratories. Two transgenic cystic fibrosis (CF) mouse models in which the ΔF508 mutation had been introduced into the CFTR gene were used: the *Cftr*^{tm1Eur} mouse (Van Doorninck et al 1995) and the *Cftr*^{tm2Cam} mouse (Colledge et al 1995). Transgenic mice were bred in the Sheffield Field Laboratories and animals 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.

Patients

Peroral jejunal biopsy specimens were obtained from 15 children (mean age 4 years 5 months; range: 2 months–15 years 11 months) with CF who were undergoing investigation for chronic diarrhoea unresponsive to changes in pancreatic enzyme supplementation. All CF patients were homozygous for the ΔF508 mutation and were pancreatic insufficient. Fifty three children (mean age 5 years 3 months; range: 2 days–14 years 4 months) under investigation for chronic diarrhoea or short stature acted as control subjects. Intestinal morphology was normal in all tissues from control and CF groups. The study was approved by the local Research Ethics Committee.

The Na^+-dependent mechanism for the absorption of glucose by the murine small intestine was first characterized using tissues from Swiss mice and its behaviour in CF was then investigated in intestinal preparations from transgenic CF mice. The effects of CF on Na^+-dependent glucose absorption were compared in human and murine tissues.

Measurement of transintestinal electrical activity

The potential difference (PD), short-circuit current (SCC) and tissue resistance were measured across paired sheets of mouse small intestine. Intact and stripped (outer muscle layers and myenteric plexus removed) tissues were used. The jejunum was taken from the region just distal to the ligament of Treitz and the mid-intestine from the mid region. 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; $H_2PO_4^-$, 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.

Jejunal biopsies were treated similarly to murine intestinal sheets but were mounted in a modified Ussing chamber with an aperture of 3 mm^2 and a 10 μA current pulse was used to assess resistance.

Murine 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 sequential additions of glucose were made to the mucosal fluid of the test sheets (with equimolar mannitol added to control sheets as an osmotic control) to give concentrations of 2.5, 5, 10, 15, 20, 25, 30, 35 and 40 mM. After each addition of glucose or mannitol the SCC was allowed to stabilize before further additions were made. The rise in SCC associated with active Na^+-dependent glucose absorption was calculated by subtracting the basal SCC from the SCC in the presence of each glucose concentration and then adding the decrease in SCC induced by the same concentration of mannitol. The apparent K_m and V_{max} values for the increase in SCC associated with active glucose absorption were calculated using a Lineweaver–Burke plot. Mannitol induced a decrease in SCC that was linearly related to its concentration and the magnitude of this effect was expressed as μA cm^{-2}/10 mM.

To compare directly the effects of removal of the outer muscle layers and the myenteric plexus on absorptive responses, adjacent sheets of mid-intestine, one intact and the other stripped, were used. After 5-min basal readings alanine or α-methyl glucoside (10 mM) was added to the mucosal solution and the increase in SCC recorded. In separate tissue pairs acetylcholine (10^{-3} M) was added to the serosal solution following 5-min basal readings. After

5-min exposure, the acetylcholine was washed out, the tissues allowed to restabilize for 5 min and then glucose (10 mM) was added to the mucosal solution after a further 5 min of basal readings. The same protocol was used to compare the responses of intact and stripped preparations of mid-intestine from wild-type and CF (*Cftr*^{tm1Eur}) mice to acetylcholine and glucose.

The effects of tetrodotoxin on the SCC responses to glucose and mannitol were investigated in intact sheets. The neurotoxin was added to the serosal solution of test sheets to give a concentration of 10^{-5} M as soon as the tissues were mounted, while control sheets received an equivalent volume (25 μL) of vehicle (154 mM NaCl).

The phenotype of the CF mice was confirmed by testing the intestinal response to secretagogue challenge. A stripped intestinal sheet was prepared from the region of mid-intestine immediately distal to that used to test the effects of glucose and mannitol and after 5-min basal readings acetylcholine (10^{-3} M) was added to the serosal solution.

To confirm the phenotype of animals used in the brush-border membrane experiments a pair of stripped sheets was prepared from the region of intestine immediately adjacent to that used for the isolation of the vesicles. After 5-min basal readings acetylcholine (10^{-3} M) was added to the serosal solution of one sheet and glucose (10 mM) was added to the mucosal solution of the other.

Human tissues were allowed to stabilize for 10 min after mounting and then readings of electrical activity were taken at 1-min intervals. Following 5-min basal readings acetylcholine (10^{-3} M) was added to the serosal solution where it remained for 10 min. It was then washed out and after a further 10 min glucose (10 mM) was added to the mucosal solution.

Measurement of Na^+-dependent glucose uptake by brush-border membrane vesicles

Brush-border membrane vesicles (BBMVs) were isolated from Swiss and transgenic CF (*Cftr*^{tm1Eur}) mice using a magnesium precipitation technique as described in detail by Klaren et al (2000). The proximal 75% intestine, measured from the ligament of Treitz, 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. The mucosa was homogenized in a 30-mL tissue grinder with a Teflon pestle (Wheaton, Millville, NJ) 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 resuspended 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 resuspended 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 concentration of approximately 1 mg protein 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 ± 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 ± 0.4-fold, n = 6).

Na^+-dependent glucose uptake was measured in duplicate using a rapid filtration protocol. BBMVs were incubated at 20 °C in a medium containing 100 μM glucose, 843 kBq mL^{-1} D-[2-^{3}H]-glucose, 0.1 mM $MgSO_4$, 20 mM HEPES/Tris, pH 7.4 with either 100 mM Na-gluconate or 100 mM K-gluconate for periods between 10 and 900 s. Uptake was terminated by adding an 18-fold surplus of ice-cold stop buffer (165 mM NaCl, 0.25 mM phlorizin, 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 × 4 mL ice-cold stop buffer and transferred to a vial, 4.5 mL Emulsifier Safe (Packard, Meriden, CT) was added and the filter allowed to dissolve. Vials were then counted in a Packard Tri-Carb 1600TR liquid scintillation counter (Packard, Meriden, CT). Glucose uptake was expressed as nmol glucose (mg protein)$^{-1}$ and Na^+-dependent glucose uptake was calculated as the difference between values obtained in the presence of Na^+ and K^+.

Statistical analysis

Results are expressed as mean values ± s.e.m. of the number of observations indicated and Student's *t*-test was used to assess significance. An unpaired *t*-test was used to compare the responses of stripped and intact intestinal sheets (Table 1); wild-type and CF mouse intestine (Tables 2 and 3); control and CF jejunal biopsies, and Na^+-dependent glucose uptake by wild-type and CF BBMVs. A direct comparison of intact and stripped preparations from adjacent regions of the same intestine was made using a paired *t*-test. A paired *t*-test was used to assess the effects of tetrodotoxin.

Results

Glucose-induced changes in transintestinal electrical activity in Swiss mice

Jejunum and mid-intestine generated a basal PD and SCC in which the serosal side of the tissue was positive with respect to the mucosal side, with values being greater in

Table 1 Basal electrical activity and responses to glucose and mannitol in intact and stripped sheets of mouse jejunum and mid-intestine. The basal potential difference (PD_b, mV), short-circuit current (SCC_b, $\mu A\,cm^{-2}$) and tissue resistance (R_b, $ohm.cm^2$) are given for tissues where either glucose or mannitol was added subsequently. Apparent K_m and V_{max} values for glucose were calculated from a Lineweaver–Burke plot and the decreases in SCC induced by the osmotic effects of mannitol (Osm) are provided.

	Jejunum		**Mid-intestine**	
	Intact (12)	Stripped (6)	Intact (7)	Stripped (9)
PD_b (glucose)	3.0 ± 0.2	1.0 ± 0.2***	2.5 ± 0.3	1.0 ± 0.1***
PD_b (mannitol)	3.6 ± 0.3	1.2 ± 0.2***	2.2 ± 0.2	1.2 ± 0.1***
SCC_b (glucose)	43.9 ± 3.7	29.1 ± 6.2*	100.4 ± 23.1	52.0 ± 5.9*
SCC_b (mannitol)	55.5 ± 6.3	36.0 ± 6.8*	77.2 ± 12.7	53.0 ± 4.2*
R_b (glucose)	70.1 ± 4.8	37.3 ± 2.0***	27.4 ± 2.8	21.3 ± 2.1
R_b (mannitol)	68.7 ± 5.8	33.3 ± 2.5***	31.0 ± 3.8	22.9 ± 1.2*
K_m (mM)	17.8 ± 2.7	15.4 ± 3.4	10.3 ± 1.9	10.9 ± 1.8
V_{max} ($\mu A\,cm^{-2}$)	84.1 ± 12.5	227.7 ± 37.6***	144.7 ± 31.5	447.0 ± 48.9***
Osm ($\mu A\,cm^{-2}$/10 mM)	7.7 ± 0.9	11.5 ± 1.3*	10.0 ± 1.7	21.4 ± 1.0***

Values are mean ± s.e.m. of the number of tissues indicated. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 2 Basal electrical activity and responses to glucose and mannitol in stripped sheets of jejunum and mid-intestine from wild-type and CF (*Cftr*^{tm1Eur}) mice. The basal potential difference (PD_b, mV), short-circuit current (SCC_b, $\mu A\,cm^{-2}$) and tissue resistance (R_b, $ohm.cm^2$) are given for tissues where either glucose or mannitol was added subsequently. Apparent K_m and V_{max} values for glucose were calculated from a Lineweaver–Burke plot and the decreases in SCC induced by the osmotic effects of mannitol (Osm) are provided. Acetylcholine (ACh, 10^{-3} M) was used to confirm the phenotype.

	Jejunum		**Mid-intestine**	
	Wild-type (7)	CF (8)	Wild-type (10)	CF (10)
PD_b (glucose)	1.8 ± 0.4	0.2 ± 0.1**	0.6 ± 0.2	0.7 ± 0.1
PD_b (mannitol)	1.5 ± 0.2	0.1 ± 0.2***	0.8 ± 0.2	0.7 ± 0.1
SCC_b (glucose)	73.9 ± 17.8	10.2 ± 4.6**	26.3 ± 6.2	24.5 ± 4.3
SCC_b (mannitol)	57.9 ± 8.6	3.1 ± 5.9***	28.9 ± 7.4	22.0 ± 4.4
R_b (glucose)	27.2 ± 2.0	26.5 ± 2.2	23.5 ± 1.4	31.9 ± 3.7*
R_b (mannitol)	27.4 ± 2.9	30.4 ± 0.9	26.2 ± 2.0	34.9 ± 4.1*
ACh ($\mu A\,cm^{-2}$)			156.6 ± 26.7	9.8 ± 1.6***
K_m (mM)	5.4 ± 0.9	4.9 ± 0.7	9.8 ± 2.2	10.1 ± 0.8
V_{max} ($\mu A\,cm^{-2}$)	342.2 ± 23.5	190.4 ± 29.4**	490.0 ± 42.9	306.0 ± 46.8**
Osm ($\mu A\,cm^{-2}$/10 mM)	12.5 ± 1.7	11.7 ± 1.1	19.0 ± 1.2	12.9 ± 2.0*

Values are mean ± s.e.m. of the number of tissues indicated. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

intact preparations (Table 1). Removal of the muscle layers reduced the tissue resistance by approximately 50% in the jejunum, with a much smaller reduction being observed in the mid-intestine (Table 1).

Glucose increased the SCC in intact and stripped sheets of jejunum and mid-intestine, but these effects were much greater in stripped preparations (Figure 1). Addition of a solute to one side of an intestinal preparation imposes an osmotic gradient that leads to the development of an electrokinetic potential and a consequent reduction in SCC (Smyth & Wright 1966), and this effect was also greater in stripped preparations (Figure 1, Table 1). Calculation of the K_m and V_{max} values revealed that the increased responses to glucose observed in the stripped preparations was due to a greater maximum transport rate.

The greater absorptive SCC induced by glucose in stripped sheets was observed with other actively transported nutrients. In sheets of mid-intestine 10 mM alanine increased the SCC by $203.5 \pm 22.8\,\mu A\,cm^{-2}$ in stripped sheets and by $96.9 \pm 17.4\,\mu A\,cm^{-2}$ in intact sheets ($n = 9$, $P < 0.01$). Equivalent values for α-methyl glucoside, an actively transported but non-metabolized hexose, were 160.2 ± 16.9 and $42.8 \pm 11.6\,\mu A\,cm^{-2}$ ($n = 6$, $P < 0.01$) and for glucose were 138.4 ± 12.7 and $37.4 \pm 8.7\,\mu A\,cm^{-2}$ ($n = 8$, $P < 0.001$), for stripped and intact sheets respectively. The effect of acetylcholine was also greater in

Table 3 Basal electrical activity and responses to glucose and mannitol in intact sheets of mid-intestine from wild-type and CF (*Cftr*^{tm2Cam}) mice. The basal potential difference (PD_b, mV), short-circuit current (SCC_b, $\mu A\,cm^{-2}$) and tissue resistance (R_b, ohm.cm^2) are given for tissues where either glucose or mannitol was added subsequently. Apparent K_m and V_{max} values for glucose were calculated from a Lineweaver–Burke plot and the decreases in SCC induced by the osmotic effects of mannitol (Osm) are also provided. Acetylcholine (ACh, 10^{-3} M) was used to confirm the phenotype.

	Wild-type (5)	CF (4)
PD_b (glucose)	1.5 ± 0.2	1.6 ± 0.1
PD_b (mannitol)	2.0 ± 0.2	1.0 ± 0.1**
SCC_b (glucose)	66.1 ± 10.1	55.5 ± 11.9
SCC_b (mannitol)	85.5 ± 14.0	33.8 ± 6.8*
R_b (glucose)	24.0 ± 5.0	32.3 ± 4.6
R_b (mannitol)	25.8 ± 2.8	33.3 ± 5.6
ACh (glucose) ($\mu A\ cm^{-2}$)	83.4 ± 23.7	-7.7 ± 1.3*
ACh (mannitol) ($\mu A\,cm^{-2}$)	49.2 ± 8.8	-10.4 ± 2.5***
K_m (mM)	15.7 ± 3.2	3.9 ± 0.4*
V_{max} ($\mu A\,cm^{-2}$)	189.6 ± 51.9	36.3 ± 4.9*
Osm ($\mu A\,cm^{-2}$/10 mM)	8.1 ± 1.9	1.9 ± 0.5*

Values are mean ± s.e.m. of the number of tissues indicated. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

stripped preparations (stripped: $118.6 \pm 7.0\ \mu A\,cm^{-2}$; intact: $18.6 \pm 8.0\ \mu A\,cm^{-2}$ (n = 8, $P < 0.001$).

The difference between intact and stripped sheets could not be attributed to loss of neural activity following removal of the myenteric plexus, as in intact sheets tetrodotoxin (TTX) had no significant effect on basal PD, SCC and R (resistance) ($P > 0.05$ in all cases). It also failed to influence the apparent K_m (control: 16.3 ± 5.5 mM; +TTX: 12.6 ± 2.0 mM, n = 7, $P > 0.05$) or the apparent V_{max} (control: $246.3 \pm 36.8\ \mu A\,cm^{-2}$; +TTX: $291.5 \pm 36.0\ \mu A\,cm^{-2}$, n = 7, $P > 0.05$). The osmotic effect of mannitol was unaffected (control: $13.2 \pm 3.1\ \mu A\,cm^{-2}$/10 mM; +TTX: $16.1 \pm 3.4\ \mu A\,cm^{-2}$/10 mM, n = 7, $P > 0.05$).

Glucose-induced changes in transintestinal electrical activity in CF mice

The basal PD and SCC were significantly lower in stripped sheets of CF jejunum compared with wild-type tissues from *Cftr*^{tm1Unc} mice, although in the mid-intestine values were similar for sheets from the two groups (Table 2). In the mid-intestine tissue resistance was greater in CF tissues, but in the jejunum there was no difference between the two groups (Table 2). The genotype was confirmed by testing the response to secretagogue challenge. Wild-type tissues exhibited a large rise in SCC when acetylcholine was administered, but CF tissues produced only a small change (Table 2). Glucose increased the SCC in wild-type and CF tissues, but its effect was significantly smaller in the CF group (Figure 2, Table 2). In the mid-intestine the osmotic effect of mannitol was also reduced in CF tissues.

The effects of acetylcholine and glucose were compared in intact and stripped sheets of mid-intestine taken from the same wild-type and CF (*Cftr*^{tm1Unc}) mice. In wild-type tissues acetylcholine increased the SCC by $228.9 \pm 30.9\ \mu A\,cm^{-2}$ in stripped sheets and by $65.8 \pm 7.5\ \mu A\,cm^{-2}$ in intact sheets (n = 7, $P < 0.001$). In CF tissues equivalent values were $1.9 \pm 0.7\ \mu A\,cm^{-2}$ in stripped sheets and $-1.4 \pm 2.6\ \mu A\,cm^{-2}$ in intact sheets (n = 8, $P > 0.05$), both being significantly lower than those observed in wild-type tissues ($P < 0.001$ in both cases). The response to glucose was greater also in stripped preparations of wild-type and CF tissues (wild-type: stripped $265.6 \pm 40.8\ \mu A\,cm^{-2}$, intact $44.6 \pm 9.0\ \mu A\,cm^{-2}$, n = 7, $P < 0.001$; CF: stripped $127.9 \pm 15.9\ \mu A\,cm^{-2}$, intact $18.5 \pm 4.7\ \mu A\,cm^{-2}$, n = 8, $P < 0.001$). In stripped ($P < 0.01$) and intact ($P < 0.05$) tissues the effect of glucose was significantly lower in the CF group.

The effects of glucose were tested in intact sheets of mid-intestine from *Cftr*^{tm2Cam} mice. In this transgenic mouse model a very similar pattern of results was obtained (Figure 3, Table 3), with glucose having a reduced effect in CF tissues. The phenotype was confirmed by the positive response to acetylcholine in wild-type tissues, with no significant effect in the CF group.

Transintestinal electrical activity in jejunal biopsies from human subjects

Basal PD and SCC values were significantly lower in CF jejuna (0.3 ± 0.1 mV, $10.8 \pm 4.3\ \mu A\,cm^{-2}$, n = 15) compared with controls (1.7 ± 0.1 mV, $58.7 \pm 3.8\ \mu A\,cm^{-2}$, n = 53; $P < 0.001$ in both cases), although tissue resistance values were similar in the two groups (control: 30.9 ± 2.0 ohm.cm^2; CF: 28.3 ± 2.5 ohm.cm^2, $P > 0.05$). Acetylcholine increased the SCC by $38.1 \pm 3.9\ \mu A\,cm^{-2}$ in control tissues, but in CF tissues it failed to have any effect ($-0.6 \pm 0.4\ \mu A\,cm^{-2}$, $P < 0.001$). Glucose increased the SCC in both groups, but in contrast to its effects in CF mice, the response was significantly greater in CF tissues (control: $24.5 \pm 2.8\ \mu A\,cm^{-2}$; CF: $40.0 \pm 4.4\ \mu A\,cm^{-2}$, $P < 0.01$).

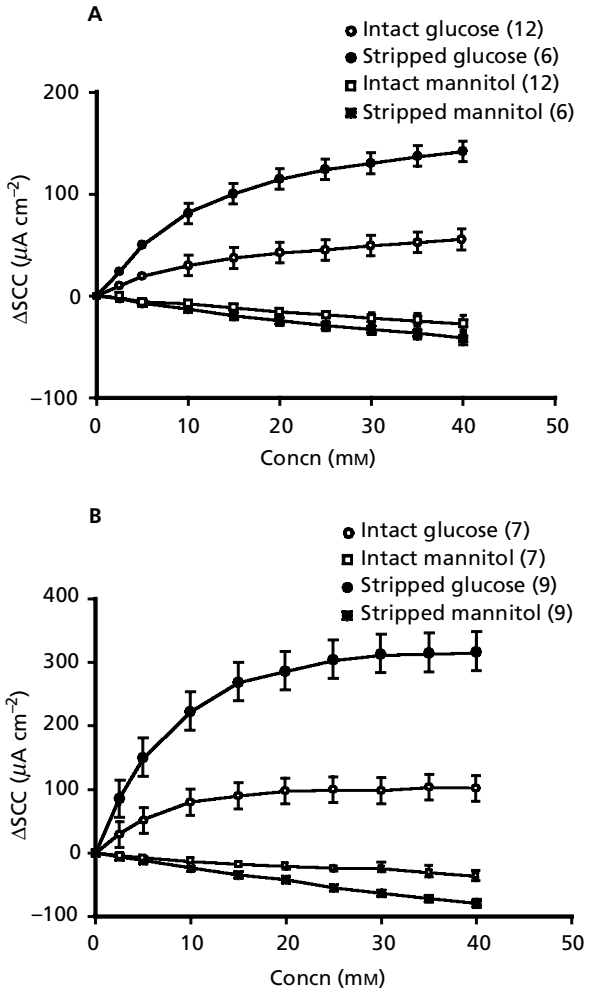

Figure 1 Changes in SCC induced by glucose and mannitol in intact and stripped sheets of jejunum (A) and mid-intestine (B) from Swiss mice. Glucose or mannitol was added cumulatively to give the concentrations shown. Each point represents the mean ± s.e.m. of the number of observations indicated.

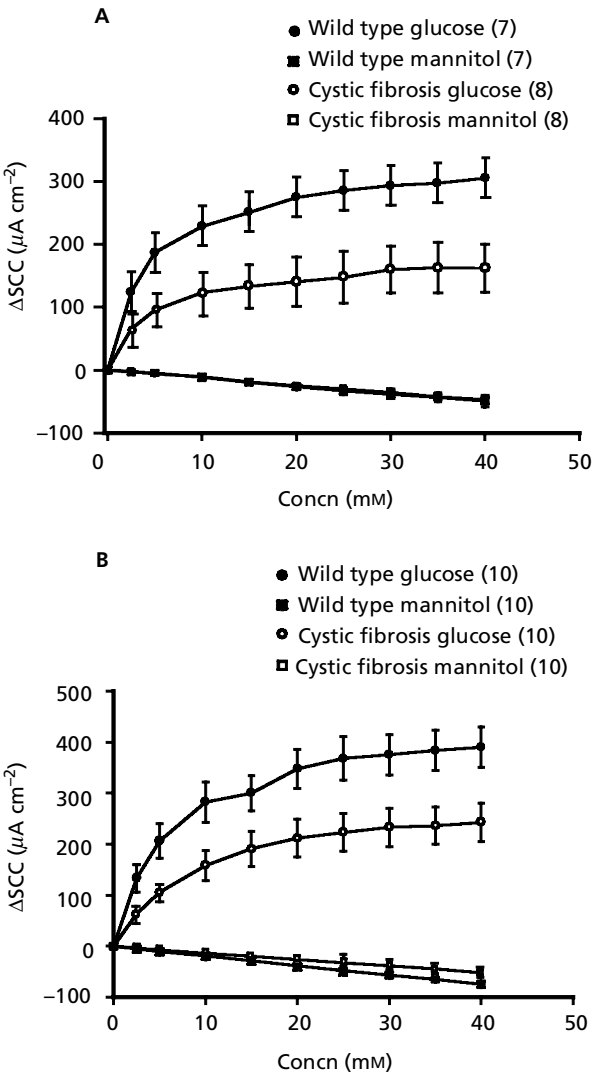

Figure 2 Changes in SCC induced by glucose and mannitol in stripped sheets of jejunum (A) and mid-intestine (B) from transgenic (*Cftr*^{tm1Eur}) wild-type and cystic fibrosis mice. Glucose or mannitol was added cumulatively to give the concentrations shown. Each point represents the mean ± s.e.m. of the number of observations indicated.

Na^+-dependent glucose uptake by brush-border membrane vesicles in Swiss mice

In the presence of an inwardly-directed Na^+ gradient an overshoot of glucose uptake was observed, after which the glucose content of the vesicles fell to an equilibrium value (Figure 4A). When Na^+ was replaced by K^+ this overshoot was not observed (Figure 4A). A Na^+-dependent overshoot was indicative of active Na^+-linked glucose uptake (Figure 4B). Peak Na^+-dependent glucose uptake was 2.6 ± 0.9 nmol (mg protein)$^{-1}$ and occurred at 45.0 ± 13.8 s (n = 7).

Na^+-dependent glucose uptake by brush-border membrane vesicles in CF mice

BBMVs were prepared from the small intestine of wild-type and CF (*Cftr*^{tm1Eur}) mice. The time course of glucose uptake was similar to that observed in BBMVs from Swiss mice and a Na^+-dependent overshoot was observed in wild-type and CF vesicles. In wild-type vesicles peak Na^+-dependent glucose uptake was 1.1 ± 0.1 nmol (mg protein)$^{-1}$ and occurred at 30.0 ± 0.0 s (n = 4), while in CF vesicles equivalent values were 1.6 ± 0.3 nmol (mg protein)$^{-1}$ and 39.5 ± 8.8 s (n = 6). Neither the peak glucose uptake nor the time at which it occurred differed between wild-type and CF vesicles ($P > 0.05$ in both cases). The phenotypes of the animals used were confirmed by the responses of stripped intestinal sheets from the same mice to acetylcholine (wild-type: $114.7 \pm 16.2\ \mu A\ cm^{-2}$; CF: $7.2 \pm 0.6\ \mu A\ cm^{-2}$, $P < 0.001$) and glucose (wild-type: $230.8 \pm 28.7\ \mu A\ cm^{-2}$; CF: $154.7 \pm 10.5\ \mu A\ cm^{-2}$, $P < 0.05$).

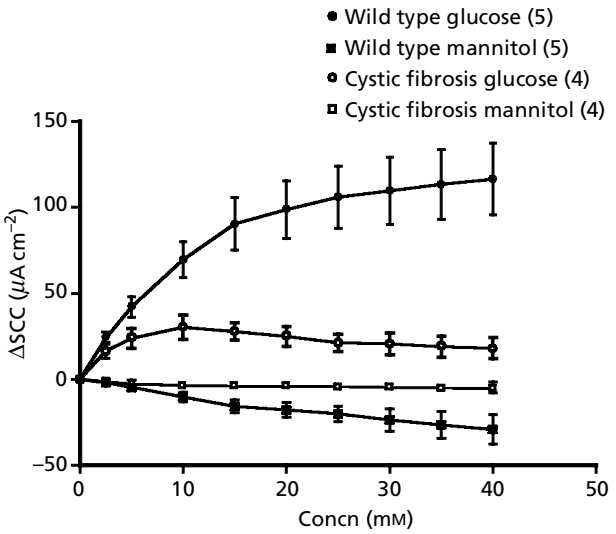

Figure 3 Changes in SCC induced by glucose and mannitol in intact sheets of mid-intestine from transgenic (*Cftr*^{tm2Cam}) wild-type and cystic fibrosis mice. Glucose or mannitol was added cumulatively to give the concentrations shown. Each point represents the mean ± s.e.m. of the number of observations indicated.

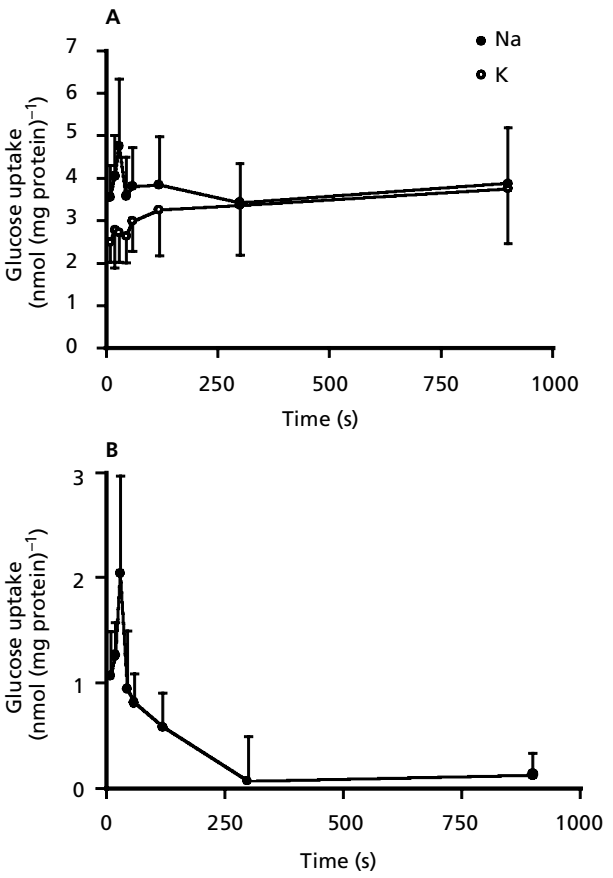

Figure 4 Time course of glucose uptake by brush-border membrane vesicles from the proximal intestine of Swiss mice. Glucose (100 μM) uptake was measured with either Na^+ or K^+ in the extravesicular medium (A) and Na^+-dependent uptake (B) was calculated as the difference between values in the presence and absence of Na^+. Each point represents the mean ± s.e.m. of seven observations.

Discussion

The first part of this study used intestinal tissues from Swiss mice to characterize Na^+-dependent glucose absorption by the jejunum and mid-intestine. In intestinal sheets from both regions glucose caused a concentration-dependent rise in SCC that previous studies had shown to be directly related to the rate of active glucose absorption (Luppa et al 1987). An unexpected finding was the much greater glucose-induced increase in SCC observed in the stripped preparation. This has not been observed in rat small intestine (Hardcastle & Hardcastle 1997). SCC rises induced by other actively transported nutrients (α-methyl glucoside and alanine) were also greater in stripped sheets. This could not be attributed to the loss of neural activity that resulted from removal of the myenteric plexus as the neurotoxin, tetrodotoxin, did not enhance the effect of glucose in intact sheets. A kinetic analysis of the glucose-dependent SCC indicated that the mid-intestine possessed a greater capacity for absorption than the jejunum. This was in agreement with a study by Madge (1972), where glucose transport by mouse small intestine was measured directly. Measurement of glucose uptake by brush-border membrane vesicles demonstrated the dependence of the transport system on the Na^+ gradient.

The secretory response to acetylcholine was also greater in the stripped preparation. However, it was added to the serosal side of the preparation and so had to penetrate a greater barrier to diffusion and was exposed to the high levels of cholinesterase present in the muscle layers and myenteric plexus (Ambache et al 1971). Both of these factors would reduce the effective concentration of acetylcholine at the basolateral membrane of the enterocyte.

Tissues from transgenic CF mice and from patients with the disease were used to investigate intestinal transport abnormalities associated with cystic fibrosis. In Ussing chamber studies, stripped sheets of mouse jejunum and human jejunal biopsies showed basal PDs and SCCs that were lower in the CF groups. These results confirmed data obtained in murine (Clarke et al 1992; Grubb 1995; Seidler et al 1997) and human (Taylor et al 1988; O'Loughlin et al 1991) tissues. This was attributed to reduced basal Cl^- secretion by CF intestine. However, lower basal values were not observed in either intact or stripped sheets of CF mouse mid-intestine. This may reflect differences in electrogenic ion transport processes along the gut. Tissue resistance in the jejunum did not differ between control and CF groups, but in the mid-intestine it was greater in CF tissues. Increased tissue resistance in CF has been reported also in human duodenal (Pratha et al 2000) and rectal (Mall et al 1999) biopsies and in mouse small intestine (Rozmahel et al 1997).

Murine and human CF tissues failed to respond to secretagogue challenge, with acetylcholine producing little

or no increase in SCC. This secretory defect is a characteristic feature of the disease and has been reported for human (Taylor et al 1987, 1988; Berschneider et al 1988; O'Loughlin et al 1991) and mouse (Clarke et al 1992; Grubb 1995) intestine.

The addition of mannitol to the mucosal solution imposes an osmotic gradient across the intestine, leading to the development of an electrokinetic potential (Smyth & Wright 1966). In jejunum and mid-intestine this effect was greater in stripped sheets than in intact preparations. In CF there were variable effects on the electrokinetic potential. In mouse jejunum there was no difference between values from wild-type and CF tissues, but in intact and stripped sheets of mid-intestine a decrease was observed in CF. This is in contrast to the increased electrokinetic response observed in human jejunal biopsies (Baxter et al 1990). An increase in passive Cl^- absorption in CF has recently been reported in human jejunum in-vivo (Russo et al 2003) and it was suggested that this could result from an alteration in charged groups within the tight junctions. An increased net negative charge would explain both the reduced passive Cl^- permeability and an increased electrokinetic potential.

In control and CF tissues glucose increased the SCC, but the effects of CF on this response differed in mouse and human tissues. In human tissues the glucose response was enhanced in the CF group and this effect has been shown to result from an increase in the maximum capacity of the transport process, as reflected in a greater apparent V_{max} value (Baxter et al 1990). In-vivo perfusion studies have demonstrated an enhanced intestinal absorption of glucose in CF patients, but this was attributed to reduced diffusion barriers (Frase et al 1985). In contrast, a recent in-vivo perfusion study in human jejunum (Russo et al 2003) failed to detect any difference in glucose absorption between control and CF groups, but the technique did not distinguish between the active and passive components of glucose absorption. It is interesting to note that in this investigation the glucose-dependent PD (associated with active glucose absorption) was greater in CF patients. Previous measurements of the glucose-dependent SCC have not detected any significant difference between control and CF tissues (Taylor et al 1987, 1988; Berschneider et al 1988; O'Loughlin et al 1991), but in each of these reports the number of patients investigated was small. In contrast to the data obtained in human tissues, the intestine from CF mice exhibited a significantly reduced SCC response to glucose. This was evident in stripped jejunum and mid-intestine from *Cftr*^{tm1Eur} mice and in intact mid-intestine from *Cftr*^{tm2Cam} mice. A kinetic analysis revealed that the apparent V_{max} value was substantially lower in CF tissues. Apparent K_m values were unchanged in stripped tissues, but in intact sheets a decrease was observed in CF. It is important to remember, however, that the apparent K_m can be influenced by changes in the V_{max} as:

$$\text{apparent } K_m = \text{real } K_m + V_{max}d/D$$

where d is the thickness of the unstirred layer and D is the free diffusion coefficient of glucose (Levin 1979). An unchanged apparent K_m in the presence of a lower apparent V_{max} in stripped preparations suggests that the real K_m may have increased, reflecting a reduced affinity of the cotransporter for its substrate. In intact preparations apparent K_m and V_{max} values were reduced to a similar extent (by 76% and 81%, respectively), so there may have been little change in the real K_m. Alternatively, abnormal mucoid secretion in CF (Eggermont 1985) could alter both the thickness of the unstirred layer (d) or the free diffusion coefficient (D), but there is currently no information on this point. It is therefore impossible to draw any conclusions regarding the effect of CF on the affinity of the Na^+/glucose cotransporter for glucose. A decreased SCC response to glucose has not been observed previously in intestinal tissues from transgenic CF mice (Clarke et al 1992; Grubb 1995; Seidler et al 1997; Joo et al 1998). However, all these studies used knockout mice in which the CF gene was disrupted, whereas our experiments used mice homozygous for the ΔF508 mutation where aberrant CFTR is produced, but due to a trafficking defect it is retained within the Golgi apparatus (Riordan 1993).

CFTR, the protein product of the CF gene, was considered to be confined to the enterocytes of the crypts originally (Crawford et al 1991; Strong et al 1994), with little being detected in the villous cells, the location of SGLT1, the Na^+/glucose cotransporter (Hwang et al 1991). However, more recent immunohistochemical localization studies in human (O'Loughlin et al 1996) and mouse (Ameen et al 2000) intestine have demonstrated that CFTR is also present in the luminal membrane of villous cells, thus raising the possibility that the two transport proteins could interact with one another.

Although the Ussing chamber experiments indicated that active glucose absorption was altered in CF, direct measurement of glucose uptake by BBMVs from the intestine of wild-type and CF mice failed to detect any difference between the two groups. It has been shown that glucose uptake by BBMVs from human intestinal biopsies is not altered in CF (Beesley et al 1996). Although CFTR is present in the brush-border membrane it may not be active in the vesicle preparation due to the loss of intracellular components such as ATP and protein kinase A that are required for its activation (Riordan 1993). When activated, normal CFTR acts as a Cl^- channel and it has been shown in rat and human BBMVs that an outwardly-directed Cl^- gradient reduces Na^+-dependent glucose uptake (Beesley et al 1997). This effect was not observed in CF vesicles. Loss of this inhibition could explain the increased glucose uptake observed in tissues from patients with CF.

From this study it was clear that CF had different effects on the glucose-dependent SCC in human and mouse intestine, but in both species individuals homozygous for the ΔF508 mutation exhibited alterations in the glucose-dependent rise in SCC. It is known that SGLT1, the cotransporter responsible for active Na^+-dependent glucose uptake, is regulated by protein kinases and species differences have been reported for the actions of protein kinase C (Wright et al 1997). This kinase reduces the activity of rabbit and rat SGLT1, but increases that of human SGLT1 when the cotransporters are expressed in *Xenopus* oocytes. It is therefore possible that CFTR can influence the behaviour of

SGLT1, but that its effects vary between species. Thus transgenic mouse models of CF may not accurately reflect all aspects of intestinal dysfunction in human patients.

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