A functional CFTR protein is required for mouse intestinal cAMP-, cGMP- and Ca^{2+}-dependent HCO_3^- secretion

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1. Most segments of the gastrointestinal tract secrete HCO_3^-, but the molecular nature of the secretory mechanisms has not been identified. We had previously speculated that the regulator for intestinal electrogenic HCO_3^- secretion is the cystic fibrosis transmembrane regulator (CFTR) channel. To prove this hypothesis, we have now measured HCO_3^- secretion by pH-stat titration, and recorded the electrical parameters of *in vitro* duodenum, jejunum and ileum of mice deficient in the gene for the CFTR protein ('CF-mice') and their normal littermates.

2. Basal HCO_3^- secretory rates were reduced in all small intestinal segments of CF mice. Forskolin, PGE_2, 8-bromo-cAMP and VIP (cAMP-dependent agonists), heat-stable enterotoxin of *Escherichia coli* (STa), guanylin and 8-bromo-cGMP (cGMP-dependent agonists) and carbachol (Ca^{2+} dependent) stimulated both the short-circuit current (I_{sc}) and the HCO_3^- secretory rate ($J_{HCO_3^-}$) in all intestinal segments in normal mice, whereas none of these agonists had any effect on $J_{HCO_3^-}$ in the intestine of CF mice.

3. To investigate whether Cl^-–HCO_3^- exchangers, which have been implicated in mediating the response to some of these agonists in the intestine, were similarly active in the small intestine of normal and CF mice, we studied Cl^- gradient-driven $^{36}Cl^-$ uptake into brush-border membrane (BBM) vesicles isolated from normal and CF mouse small intestine. Both the time course and the peak value for 4,4′-diisothiocyanostilbene-2′,2-disulphonic acid (DIDS)-inhibited $^{36}Cl^-$ uptake was similar in normal and CF mice BBM vesicles.

4. In summary, the results demonstrate that the presence of the CFTR channel is necessary for agonist-induced stimulation of electrogenic HCO_3^- secretion in all segments of the small intestine, and all three intracellular signal transduction pathways stimulate HCO_3^- secretion exclusively via activation of the CFTR channel.

HCO_3^- secretion is now known to occur in the stomach, the small and the large intestine (Dietz & Field, 1973; Brown, Parsons & O'Grady, 1992; Allen, Flemström, Garner & Kivilaakso, 1993; Minhas, Sullivan & Field, 1993; Dagher, Rho & Charney, 1993), and is a necessary component in the maintenance of the 'microclimate' near the apical membranes of gastric and intestinal surface cells. Particularly high secretory rates have been found in the proximal duodenum (Flemström, Garner, Nylander, Hurst & Heylings, 1982), where the HCO_3^- ions in part neutralize the gastric acid entering its lumen. Intestinal HCO_3^- secretion has been studied in greatest detail in the duodenum. Duodenal HCO_3^- secretion is thought to be mediated in part by electroneutral Cl^-–HCO_3^- exchange, in part by an electrogenic secretory pathway, and in part by paracellular diffusion (Allen *et al.* 1993; Isenberg, Ljungstrom, Säfsten & Flemström, 1993). Different agonists are thought to stimulate secretion via activation of different secretory transport pathways (Flemström & Garner, 1982; Heylings & Feldman, 1988; Allen *et al.* 1993; Yao, Hogan, Bukhave, Koss & Isenberg, 1993), of which none has been characterized at a molecular level.

Duodenal electrogenic HCO_3^- secretion is proportionally greater than electroneutral HCO_3^- secretion (Flemström, Heylings & Garner, 1982) and is the secretory pathway activated by most secretagogues (Allen *et al.* 1993; Guba, Kuhn, Forssmann, Classen, Gregor & Seidler, 1996). Its molecular nature is unclear: possibilities are an anion channel with high HCO_3^- permeability, or a Cl^- channel functionally coupled to an anion exchanger through which the secreted Cl^- is recycled, as has been suggested for pancreatic ductal HCO_3^- secretion (Novak & Greger, 1988), or ileal and colonic HCO_3^- secretion (Hubel, 1967; Turnberg, Bieberdorf, Morawski & Fordtran, 1970; Davis,

Morawski, Santa Ana & Fordtran, 1983). The cystic fibrosis transmembrane regulator (CFTR) channel is abundantly expressed in all parts of the small intestine (Trezise & Buchwald, 1991; Strong, Boehm & Collins, 1994) and is permeable to HCO_3^-, albeit much less than to Cl^- (Gray, Pollard, Harris, Coleman, Greenwell & Argent, 1990; Poulsen, Fischer, Illek & Machen, 1994).

The present study was undertaken to find out whether the expression of a functional CFTR protein was essential to agonist-stimulated HCO_3^- secretion in all parts of the small intestine. We also wanted to know whether the CFTR protein was the common final regulator for agonists of the three different second messenger systems currently known to be involved in electrogenic HCO_3^- secretion. Lastly, we wanted to know whether the defect in intestinal HCO_3^- secretion that we observed in all small intestinal segments of CFTR (−/−) mice was related to a secondary defect in the intestinal anion exchanger. We therefore studied basal and stimulated HCO_3^- secretion in stripped duodenal, jejunal and ileal mucosa of CFTR (−/−) mice and their normal littermates in the Ussing chamber. We also prepared small intestinal brush-border membrane (BBM) vesicles from normal and CFTR (−/−) mice and compared the anion exchange activities of these vesicles. The results demonstrate that mice lacking a functional CFTR protein have a reduced basal HCO_3^- secretory rate and are refractory to agonist stimulation, and that this is not due to a secondary defect in BBM anion exchange activity.

METHODS

Animal breeding

The CF null mice mutants had been established in the laboratories of R. Ratcliff, W. H. Colledge and M. Evans and previously characterized (Ratcliff *et al.* 1993). No wild-type CFTR protein is made by the null CF mice, since the hypoxanthine phosphoribosyl transferase (HPRT) cassette disrupts the *cftr* coding sequence and introduces a termination codon, and none of the possible RNA transcripts from the disrupted locus can encode a functional CFTR protein. The mice were raised in the animal facility of the Department of Biochemistry in Tübingen under standardized light and climate conditions. The offspring were genotyped approximately 1 week after birth, after which both the −/− and the +/+ mice and their mothers were placed on a fibre-free diet (Special Diet Services, Cambridge, UK), a special bedding ('Corncob', Mucedola, Settimo Milanese, Italy), and a polyethylene glycol-containing electrolyte drinking solution (Kleanprep, Norgine, Marburg, Germany). There was a high mortality rate of the −/− mice in the first 1–3 days after birth, but the few remaining pups survived well under these special measures up to 4–6 weeks of age and weighed 10–14 g when they were used for the experiments. Longer survival times were not tested. At the time of the experiments, the −/− mice had no significant growth deficit compared with their littermates and the gut looked normal both macroscopically and under the stereomicroscope. The protocol for the animal experiments was approved by the university veterinarian and the Regierungspräsidium Tübingen according to current state law.

Genotyping of progeny by polymerase chain reaction

Genomic DNA was prepared from 3–5 mm tail samples from 1- to 2-week-old mice by the QIAamp tissue kit (Qiagen, Hilden, Germany) and 250 ng of the DNA was added to 100 μl of polymerase chain reaction (PCR) mixture: 2 U AGS *taq* DNA polymerase (Angewandte Gentechnologische Systeme (AGS), Heidelberg, Germany); AGS buffer E; 200 μM of each dNTP; 50 nM of forward and reverse primer. For the detection of the wild-type allele the oligonucleotides 5′TCCTGATGTTGATTTTGGGA3′ and 5′TGGCTGTCTGCTTCCTGACTATGG3′ were used as primers and amplified a 383 base pair (bp) fragment. The HPRT primers 5′TGCCGACCCGCAGTCCCAGCGTCG3′ and 5′CGTGGGGGTCCTTTTCACCAGC3′ amplified a 508 bp fragment of the mutant allele (reaction mix: 2 U AGS *taq* DNA polymerase; AGS reaction buffer Ac; 200 μM of each dNTP; 50 nM of forward and reverse primer; 250 ng DNA). The PCR was performed with the following temperature parameters: 40 cycles of 93 °C for 30 s, 60 °C for 1 min and 70 °C for 1 min.

Ussing chamber experiments

Mice were anaesthetized by placing them in a jar containing a few drops of ether, then killed by cervical dislocation, the abdomen opened and the gut removed from the oesophagus to the rectum. The guts were rinsed with and immersed in ice-cold oxygenated Krebs–Ringer saline, the mesentery removed and the intestinal segments to be studied cut open at the insertion of the mesentery, placed serosal side up into a preparation chamber filled with ice-cold oxygenated standard Krebs–Ringer solution (Delta-Pharma, Pfullingen, Germany) and fixed with insect needles. The serosal layer and the two muscle layers were carefully removed with dissecting forceps. The mucosa was mounted between two specially designed oval lucite half-chambers of 0·62 cm^2 exposed area and placed in an Ussing apparatus. The bathing solutions were circulated by a gas-lift system at 37 °C. The unbuffered solution (154 mmol l^{-1} NaCl) on the luminal side was circulated with a 100% O_2 gas-lift system and the nutrient solution with carbogen (95% O_2 : 5% CO_2) at pH 7·4 ± 0·03. Luminal pH was maintained at 7·4 by a continuous pH-stat titration method (Radiometer, Copenhagen) using an isotonic solution containing 5 mM HCl or H_2SO_4 (for the Cl^- substitution experiments). Tetrodotoxin (TTX, 10^{-6} M) was added to the serosal solution. After steady rates of luminal alkalization and stable electrical parameters had been recorded for a minimum of 20 min, peptides, hormones or drugs were administered. The rate of alkalization (bicarbonate secretion) was calculated from the consumption volume of the HCl-containing titrant solution required to maintain the luminal pH at 7·4. Measurements of bicarbonate secretion were continuously recorded, averaged for 5 min periods and expressed in micromoles per hour per square centimetre. The open-circuit transepithelial electrical potential difference (PD) and short-circuit current (I_{sc}) were recorded (DVC-1000 Dual Voltage Clamp, World Precision Instruments) via agar–3 M KCl bridges. The direct current electrical resistance was determined from the change of PD after sending current (40 μA cm^{-2}) through the mucosa in either direction in a 200 ms interval, every minute.

The serosal perfusate contained (mM): Na^+, 140·5; K^+, 4·5; Ca^{2+}, 2; Mg^{2+}, 1·3; Cl^-, 116; SO_4^{2-}, 1·3; HCO_3^-, 20; HPO_4^{2-}, 1·5; dextrose, 11·9; pyruvate, 10; indomethacin, 0·01. The luminal perfusate contained (mM): Na^+, 154; Cl^-, 154. The osmolarity of both solutions was 308 mosmol l^{-1}. Na^+ was replaced by tetramethylammonium and Cl^- by gluconate in equimolar concentrations. Solutions were made on the day of use or the evening before. Aliquots of

Table 1. Basal and forskolin (10 μM)-stimulated I_{sc} and $J_{HCO_3^-}$ (peak values) in duodenum, upper jejunum and distal ileum of CF mice and normal (+/+) littermates

	Duodenum		Jejunum		Ileum	
	Normal	CF mice	Normal	CF mice	Normal	CF mice
I_{sc} (μA cm^{-2})						
Basal	47 ± 14	29 ± 8	67 ± 6	47 ± 8**	54 ± 12	33 ± 10*
Forskolin stimulated	144 ± 22***	40 ± 6	227 ± 21***	66 ± 8	118 ± 19***	41 ± 11
$J_{HCO_3^-}$ (μmol h^{-1} cm^{-2})						
Basal	1·12 ± 0·11	0·64 ± 0·10**	0·88 ± 0·06	0·48 ± 0·08**	0·62 ± 0·05	0·40 ± 0·04**
Forskolin stimulated	2·2 ± 0·22**	0·59 ± 0·15	1·98 ± 0·13***	0·52 ± 0·22	0·98 ± 0·08**	0·45 ± 0·1

The stimulated values are peak values. $n = 9$ for normal duodenum, 5 for normal and 6 for CF jejunum, and 5 for normal ileum, CF duodenum and ileum. ***$P < 0·001$, **$P < 0·01$, *$P < 0·05$. Forskolin caused a small I_{sc} but not $J_{HCO_3^-}$ increase in CF mouse intestine (significant for jejunum, $P < 0·05$ with Spearman rank test). Between CF mice and their normal littermates, there was also a significant difference in basal I_{sc} in the jejunum and ileum, and in basal $J_{HCO_3^-}$ in all intestinal segments ($P < 0·01$). Statistical comparisons were performed between basal I_{sc} and $J_{HCO_3^-}$ in CF mice and normal littermates (compare values given in the rows), and between basal and stimulated I_{sc} and $J_{HCO_3^-}$ in CF mice and normal littermates, respectively (compare values in the columns). The values are not from identical preparations to those shown in Fig. 1.

maximally 150 μl were added to the nutrient or basal solution to provide final concentrations of test agents.

Preparation of intestinal BBM vesicles

Mice were anaesthetized by placing them in a jar containing an ether-soaked piece of cotton and then killed by cervical dislocation. The full length of the small intestine was opened, the mucosa gently scraped off with a glass slide and suspended in ice-cold preparation buffer (mM: mannitol, 300; Tris, 10; Hepes, 16, pH 7·4; dithiothreitol (DTT), 1; EDTA, 1; and 40 mg l^{-1} phenylmethylsulphonyl fluoride (PMSF)). All the following steps were performed at 4 °C. Homogenization was performed by a Polytron homogenizer at low speed for 30 s, followed by homogenization in a Teflon–glass homogenizer by 20 strokes at 1000 r.p.m. Further preparation was according to the method of Knickelbein *et al.* (Knickelbein, Aronson, Atherton & Dobbins, 1983). After homogenization, $CaCl_2$ was added to a final concentration of 10 mM and the suspension incubated for 15 min. Then the homogenate was centrifuged at 3000 *g* for 10 min, the pellet discarded, the supernatant centrifuged at 27 000 *g* for 60 min and the resulting pellet resuspended. Magnesium gluconate was added to the suspension at a final concentration of 10 mM and the procedure repeated twice. The final pellet was further treated and used for uptake studies the same day, as described later. Enrichment of the BBM vesicles was determined by measuring the activity of brush border and basolateral marker enzymes in the initial homogenate, the final pellet, and the membrane suspension that had been pelleted in the intravesicular buffer and revesiculated. Enrichment for the alkaline phosphatase from normal littermates ($n = 5$) and CFTR (−/−) mice ($n = 3$) was 14·6 ± 1·8-fold and 12·8 ± 3·3-fold, respectively, in the final pellet and 29·7 ± 5·6-fold and 24·2 ± 6·4-fold, respectively, in the revesiculated membrane suspension. (The higher enrichment factor corresponds to a lower protein content after centrifugation and revesiculation, so the further enrichment is probably due to another 'washing' procedure.) Na^+–K^+-ATPase activity was enriched 1·8 ± 0·9-fold and 1·2 ± 0·8-fold in normal and CFTR (−/−) mice, respectively, in the final pellet and was, for reasons not clear to us, further reduced to values below 0·5-fold in the revesiculated membrane fraction (not assayed every time).

Enzyme assays

Protein determination was performed by the method of Lowry with modifications as described by Peterson (1983). Na^+–K^+-ATPase activity as a marker enzyme for basolateral membranes was measured as described by Forbush (1983). The activity of alkaline phosphatase was used as a marker for BBMs, since, though there is a small amount of alkaline phosphatase activity on basolateral membranes, most activity is located on the BBM and enzyme activity can be assayed conveniently, and was determined by measuring the hydrolysis of *p*-nitrophenyl phosphate at pH 10·3 (Hanna, Mircheff & Wright, 1979). In addition, the activity of sucrase, as a pure BBM enzyme, was determined as described by Dahlquist (1964). Enrichment of sucrase activity paralleled that of alkaline phosphatase and was therefore not performed routinely.

Isotope flux studies

The membranes were suspended in the appropriate 'intravesicular' buffer solution and pelleted at 30 000 *g*. The pellet was revesiculated by twenty-five passes through a 25 G needle in the intravesicular buffer solution plus 0·2 mM valinomycin and the suspension incubated for 1·5 h at room temperature. A 5 μl aliquot of vesicles was added to 45 μl extravesicular buffer containing $^{36}Cl^-$. At varying time intervals, isotope uptake was stopped by the addition of 3 ml ice-cold stop solution (100 mM potassium gluconate, 60 mM mannitol, 35 mM Mes, 5 mM Tris, pH 5·5). The solution was filtered immediately through 0·45 μm Millipore HAWP filters (Millipore, Eschborn, Germany) and washed twice with 3 ml stop solution. Filters were transferred to 5 ml scintillation cocktail (Packard, Frankfurt, Germany) and radioactivity was measured using a β-scintillation counter. At each time point, triplicate measurements of $^{36}Cl^-$ uptake into BBM vesicles were performed. All media were gassed with 100% N_2 except those containing HCO_3^-, which were gassed with N_2/25% CO_2. The compositions of the intra- and extravesicular solutions are listed in detail in the legend of Fig. 7. Unless specifically mentioned, the concentration of

extravesicular Cl^- is calculated as the Cl^- concentration after mixing the extravesicular solution (containing $^{36}Cl^-$) with the vesicle suspension (containing only unlabelled Cl^-). The osmolarity of each solution was checked with an osmometer and equalized. To clamp the transvesicular membrane potential to zero, the K^+ ionophore valinomycin and equal intra- and extravesicular K^+ concentrations were used. 4,4'-Diisothiocyanostilbene-2',2-disulphonic acid (DIDS; 3 mM) was used to inhibit anion exchange. Both in the ileal (Knickelbein, Aronson, Atherton & Dobbins, 1985) and duodenal (authors' unpublished observations) BBM vesicles of rabbit intestine, such a high concentration has been found to be necessary to inhibit the anion exchange. All transport studies were performed at room temperature, which was between 20 and 24 °C, depending on the season.

Materials

Indomethacin, PGE_2 and 16,16-dimethyl-prostaglandin E_2, carbachol, 8-bromo-cyclic adenosine monophosphate (8-Br-cAMP), 8-bromo-cyclic guanosine monophosphate (8-Br-cGMP), heat-stable *Escherichia coli* enterotoxin (STa), DIDS, vasoactive intestinal

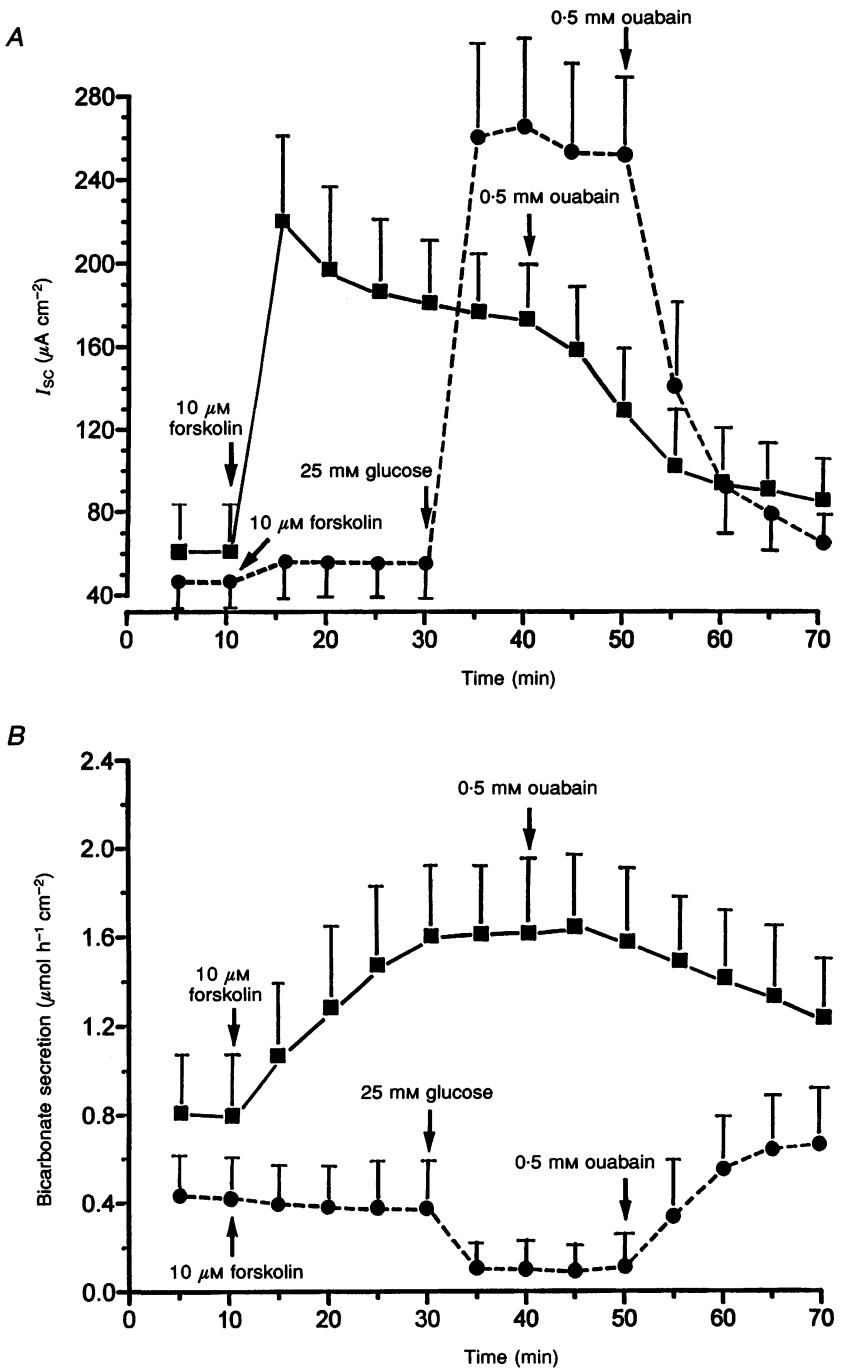

Figure 1. Time course of changes in I_{sc} and $J_{HCO_3^-}$ in normal (■) and CF mouse (●) jejunum in response to 10 μM forskolin and to 25 mM glucose in CF mouse tissue

A, changes in I_{sc}. *B*, changes in bicarbonate secretion rate ($J_{HCO_3^-}$). The respective results with glucose in normal mouse jejunum are given in the text. $n = 3$ (CF mice) and 5 (normal mice).

Table 2. Changes in I_{sc} and $J_{HCO_3^-}$ in response to a variety of cAMP-dependent agonists in duodenum, upper jejunum and distal ileum

	Duodenum		Jejunum		Ileum	
	ΔI_{sc}	$\Delta J_{HCO_3^-}$	ΔI_{sc}	$\Delta J_{HCO_3^-}$	ΔI_{sc}	$\Delta J_{HCO_3^-}$
8-Br-cAMP (1 mM)	121 ± 24	1·06 ± 0·09	147 ± 14	0·94 ± 0·05	74 ± 13	0·55 ± 0·08
VIP (100 nM)	165 ± 40	1·02 ± 0·11	187 ± 22	0·9 ± 0·06	n.d.	n.d.
PGE_2 (1 μM)	70 ± 27	0·7 ± 0·08	99 ± 13	0·64 ± 0·0	69 ± 21	0·44 ± 0·08

Only the changes in normal mouse intestine are shown, since no significant changes occurred in response to any of the agonists in CF mouse intestine. There was, however, always a very small short-lived PD peak of 0·1–0·2 mV in the CF epithelium after addition of the agonists. n.d., not done. $n = 3$–7. Units for ΔI_{sc} and $\Delta J_{HCO_3^-}$ as in Table 1.

Table 3. Changes in I_{sc} and $J_{HCO_3^-}$ in upper jejunum of normal and CF mice in response to cGMP-dependent agonists and carbachol

	ΔI_{sc}		$\Delta J_{HCO_3^-}$	
	Normal	CF mice	Normal	CF mice
8-Br-cGMP (1 mM)	102 ± 19	n.ch.	1·24 ± 0·11	n.ch.
STa (100 nM)	67 ± 11	n.ch.	0·8 ± 0·07	n.ch.
Guanylin (100 nM)	54 ± 11	n.ch.	0·71 ± 0·04	n.ch.
Carbachol (0·1 mM)	218 ± 30	−13 ± 2	0·43 ± 0·04	n.ch.

For 8-Br-cGMP, STa and guanylin stimulation, there was a tiny initial PD peak of 0·1–0·2 mV in CF epithelium that was so short-lived that usually no current pulse was registered during that time. The I_{sc} change in carbachol-treated CF epithelium was biphasic: an initial I_{sc} decrease was followed by an increase due to a decrease in resistance (see Fig. 5). n.ch., no significant change compared with unstimulated tissue. $n = 4$–7. Units for ΔI_{sc} and $\Delta J_{HCO_3^-}$ as in Table 1.

peptide (VIP), dithiothreitol (DTT), TTX and PMSF were obtained from Sigma–Aldrich (Deisenhofen, Germany). Guanylin was purchased from Saxon Biochemicals (Hannover, Germany). The buffer substances, salts and other reagents for the marker enzyme assay were purchased from Merck (Darmstadt, Germany) or Sigma–Aldrich, at analytical grade purity. $H^{36}Cl$ was purchased from Amersham (Braunschweig, Germany).

Statistics

Values are means ± S.E.M. n is the number of different animals. Statistical analysis was performed with Student's t test or the Spearman rank test for paired samples and a P value $< 0·05$ was considered significant, if the appropriate number of experiments required for statistical evaluation had been performed. Due to the limited availability of CF mice and the clarity of the data, many experiments with CF epithelium were only repeated 3–4 times.

RESULTS

Stimulation with cAMP-dependent agonists

Figure 1*A* shows the time course of changes in I_{sc} on stimulation with 10 μM forskolin in the upper jejunum of normal and CF mice. It is evident that there is a strong rise in I_{sc} upon stimulation in the normal mouse jejunum and a minimal I_{sc} increase in CF mouse jejunum. When 25 mM glucose was added luminally (to stimulate Na^+–glucose cotransport) there was a prompt rise in I_{sc} in the CF mouse jejunum, which was equivalent to that in the normal mouse jejunum (I_{sc} values of 269 ± 33 *vs.* 253 ± 29 μA cm^{-2} for CF and normal mice, respectively, $P > 0·05$), indicating that villus atrophy is not likely to be present in the CF mice. Figure 1*B* shows that a HCO_3^- secretory response upon forskolin stimulation was only seen in normal, not CF, mouse upper jejunum. The HCO_3^- secretory response was typically somewhat delayed and more sustained compared with the I_{sc}. Serosal ouabain (0·5 mM) caused a slow decrease in I_{sc} to zero (over the next 120 min) and decreased HCO_3^- secretion to about 50% of basal value in the normal mouse jejunum. In the CF mouse jejunum, the basal HCO_3^- secretion rate ($J_{HCO_3^-}$) was approximately half that seen in normal tissue ($P < 0·01$), and no stimulation was observed with forskolin. Luminal glucose (25 mM) caused a decrease of $J_{HCO_3^-}$ which was also seen in the jejunum of normal mice. The addition of 0·5 mM serosal ouabain to CF jejunum caused a transient rise in HCO_3^- secretion with a very slow decrease to approximately 20% above basal value.

The pattern of response of duodenal and ileal tissue was similar to that in the upper jejunum, except that basal and peak values were different (Table 1), possibly due to different expression of the CFTR protein (Trezise & Buchwald, 1991; Strong *et al.* 1994). Although the basal $J_{HCO_3^-}$ in the duodenum was slightly higher than in the jejunum, it was

considerably more variable, and the response to all agonists was qualitatively similar. We therefore chose to work primarily with jejunal tissue. 8-Br-cAMP, VIP and PGE_2 caused an increase in I_{sc} and $J_{HCO_3^-}$ in all parts of the intestine of normal mice and had no effect on $J_{HCO_3^-}$ in any part of the intestine of CF mice (Fig. 2 and Table 2), demonstrating that neither substance stimulated a different HCO_3^- secretory mechanism to forskolin. The I_{sc} and HCO_3^- secretory response in normal mouse intestine was more sustained with forskolin and cAMP analogues than that seen with receptor-mediated stimulation. In CF mouse intestine, forskolin and 8-Br-cAMP caused a small sustained increase in I_{sc}, whereas VIP, PGE_2 and the cGMP-dependent agonists caused a very small and short-lived PD increase of 0·1–0·2 mV and no significant change in I_{sc} after this tiny peak. In normal, but not CF mouse small intestine VIP caused a consistent rise in tissue resistance not seen with PGE_2, forskolin and 8-Br-cAMP.

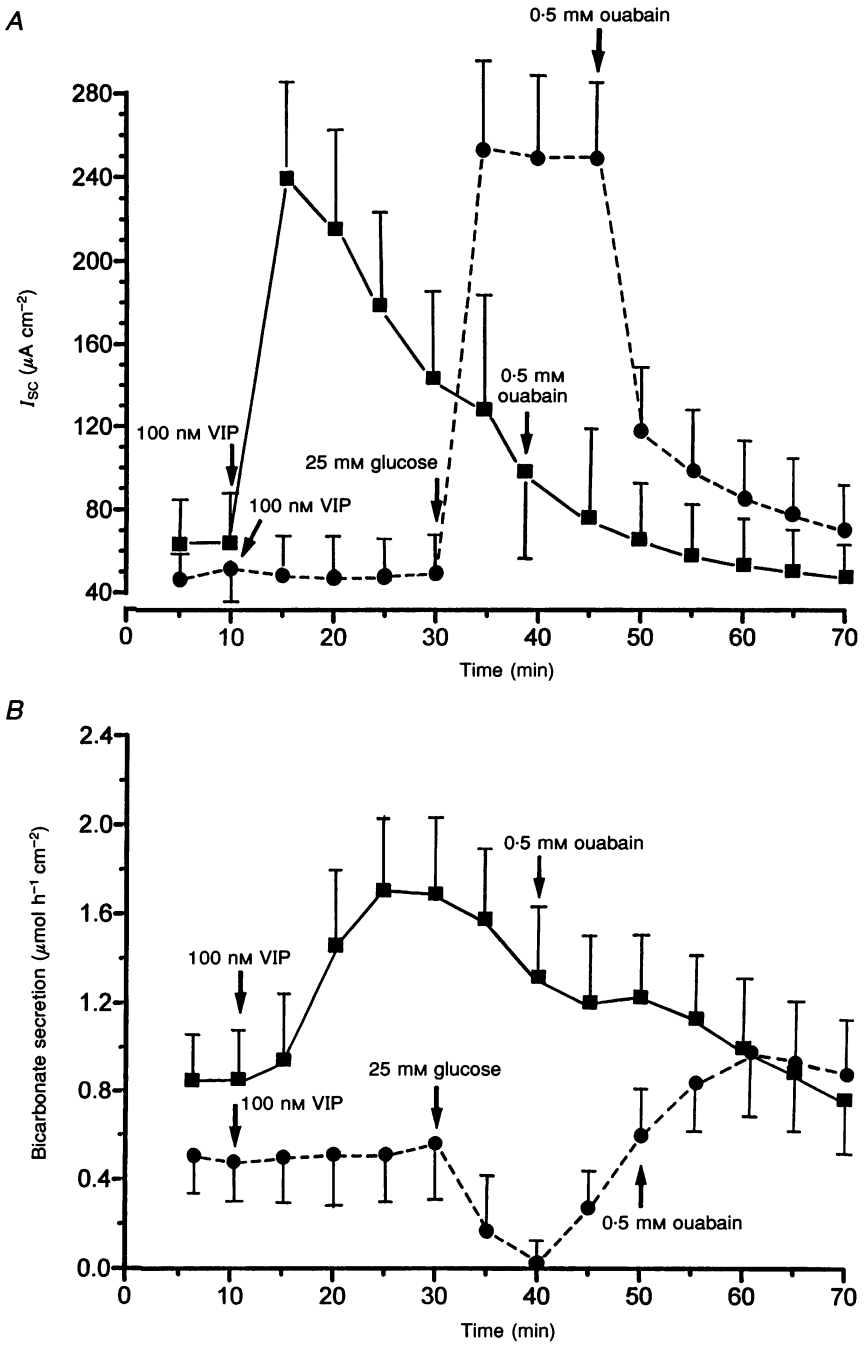

Figure 2. Time course of changes in I_{sc} and $J_{HCO_3^-}$ in normal (■) and CF mouse (●) jejunum in response to 100 nM VIP and to 25 mM glucose in the CF mouse tissue

A, changes in I_{sc}. *B*, changes in $J_{HCO_3^-}$. In comparison to the response to forskolin, the I_{sc} and HCO_3^- secretory response is transient. $n = 4–6$.

The experiments demonstrate that the basal I_{sc} and $J_{HCO_3^-}$ values are reduced in the small intestine of CF mice, and that a rise in intracellular cAMP stimulates I_{sc} as well as $J_{HCO_3^-}$ in all small intestinal segments of normal mice *in vitro*, but has no effect on $J_{HCO_3^-}$ in CF mice.

Stimulation with cGMP- and Ca^{2+}-dependent agonists

Figure 3*A* shows the effect of 1 mM 8-Br-cGMP on I_{sc} in the upper jejunum of normal and CF mice. A strong increase in I_{sc} was seen in the normal mice, with no response in the CF mice (apart from a tiny short-lived PD peak). 8-Br-cGMP also caused an increase in $J_{HCO_3^-}$ in normal mouse jejunum, with no change in CF mice (Fig. 3*B*). STa (100 nM; Fig. 4) and guanylin (100 nM) caused weaker, but qualitatively similar increases in I_{sc} and $J_{HCO_3^-}$ in normal mice, as is to be expected at these concentrations (Guba *et al.* 1996; Pfeifer *et al.* 1996), but no change in either parameter in the CF mice

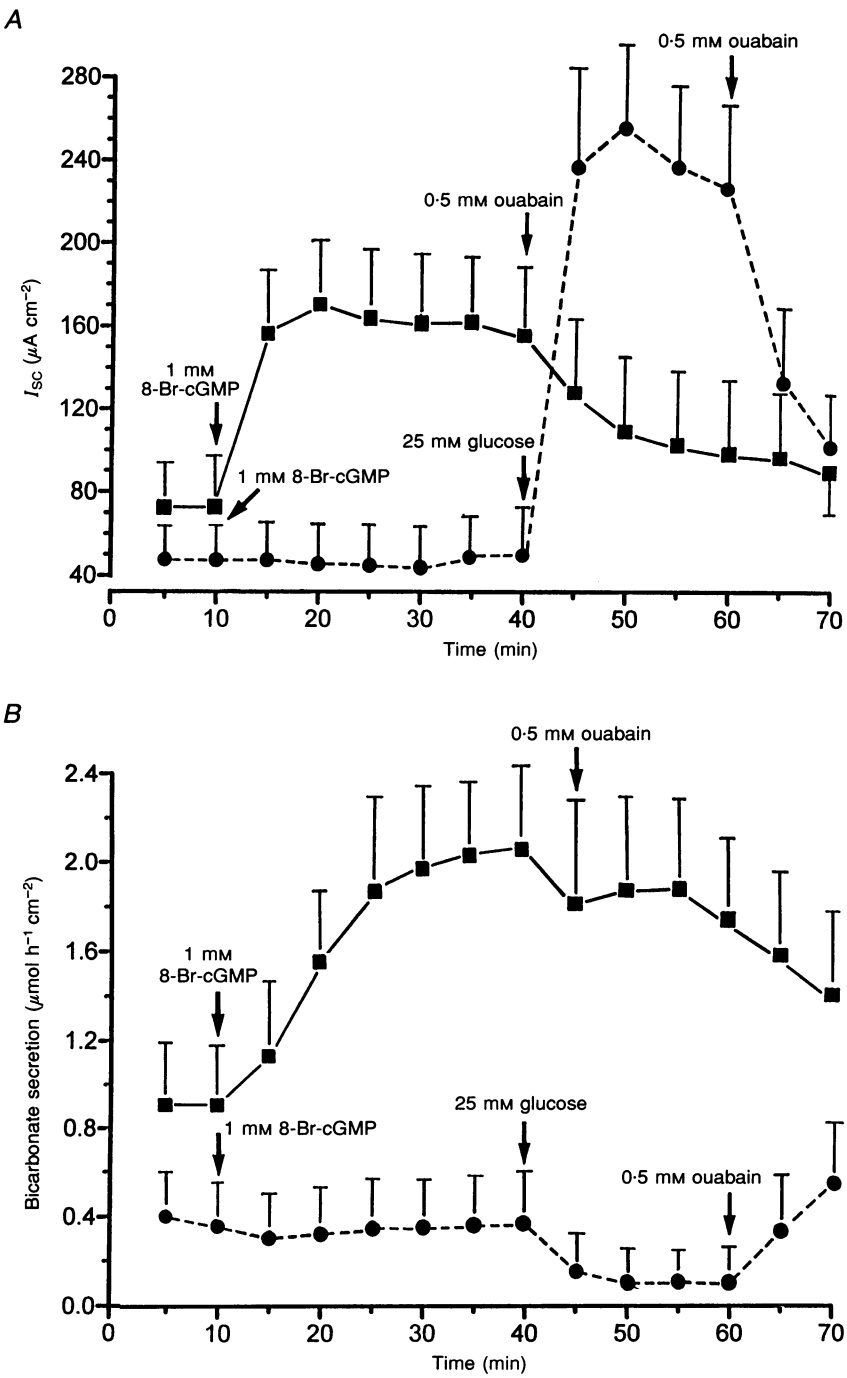

Figure 3. Time course of changes in I_{sc} and $J_{HCO_3^-}$ in normal (■) and CF mouse (●) jejunum in response to 1 mM 8-Br-cGMP and to 25 mM glucose in the CF mouse tissue

A, changes in I_{sc}. *B*, changes in $J_{HCO_3^-}$. $n = 4$–6.

apart from a tiny short-lived PD peak (Table 3). Carbachol (100 μM) caused a transient strong increase in I_{sc} and a rather weak stimulation of $J_{HCO_3^-}$ in normal mice (Fig. 5). In CF mice, there was no HCO_3^- secretory response and the I_{sc} response was biphasic. There was a small, short-lived decrease of PD resulting in a decrease in I_{sc}, then a return of the PD to prestimulation values accompanied by a decrease in tissue resistance, which resulted in a small and more sustained I_{sc} increase.

Again, the I_{sc} and HCO_3^- secretory response was more sustained with the cGMP analogue than with receptor-mediated stimulation, and was particularly short with carbachol, as has been observed previously in rat duodenum (Guba *et al.* 1996). The ratio of I_{sc} to $J_{HCO_3^-}$ was higher for the cAMP-dependent agonists compared with the cGMP-dependent agonists, as has also been observed in rat duodenum (Guba *et al.* 1996). These results were qualitatively similar in the duodenum and ileum.

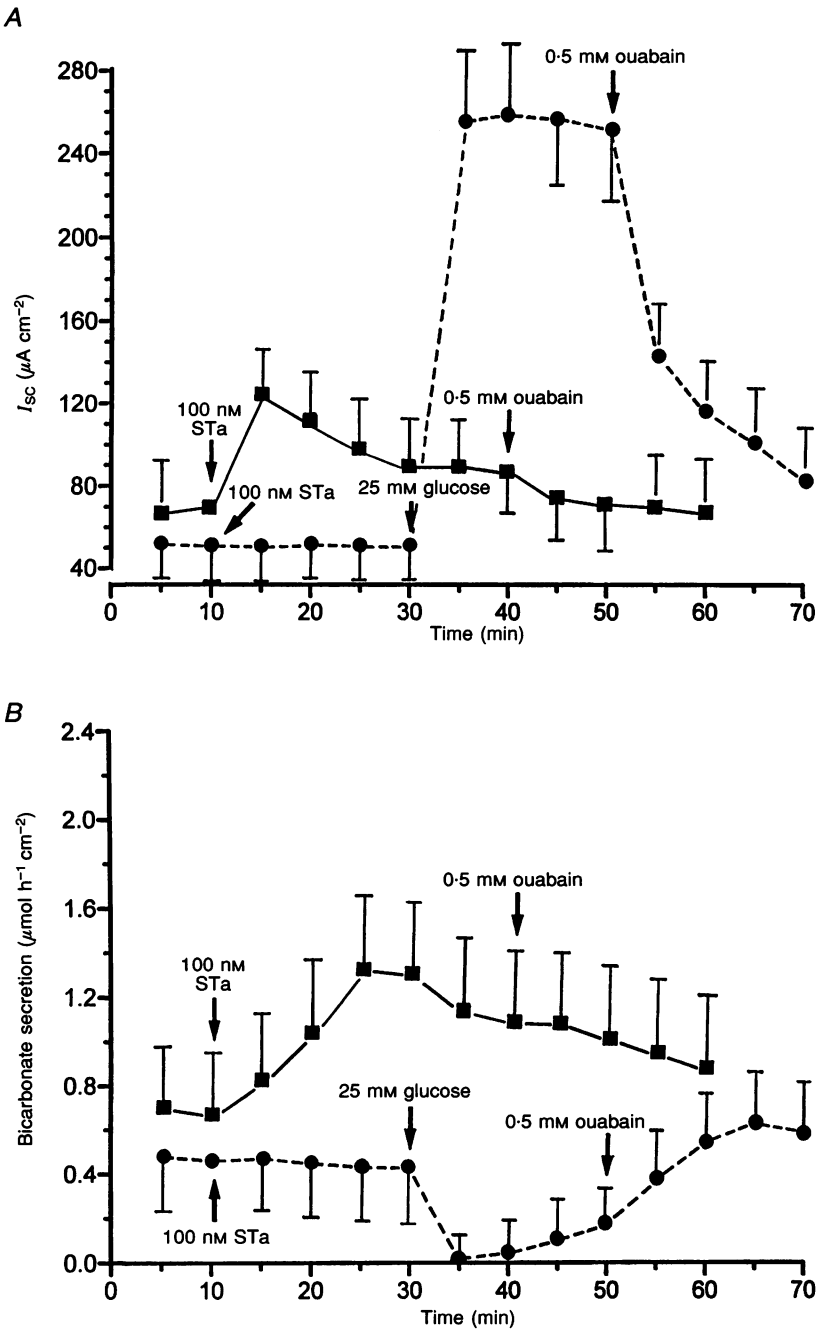

Figure 4. Time course of changes in I_{sc} and $J_{HCO_3^-}$ in normal (■) and CF mouse (●) jejunum in response to 100 nM STa and to 25 mM glucose in the CF mouse tissue

A, changes in I_{sc}. *B*, changes in $J_{HCO_3^-}$. $n = 4$–6.

Intestinal Cl^-–HCO_3^- exchange in normal and CF mice

It is presently not clear how the HCO_3^- ion is secreted into the lumen during electrogenic HCO_3^- secretion, and one possibility is that CFTR channel activation provides the driving force for HCO_3^- exit in exchange for Cl^- entry, caused by a decrease in $[Cl^-]_i$. It was therefore necessary to study anion exchange in the small intestine of CF mice and compare it with that in normal mice. Two different experimental protocols were used: (1) removal of Cl^- from the luminal solution (which should *decrease* HCO_3^- secretion mediated by anion exchange), and (2) measuring the $^{36}Cl^-$ uptake rate into small intestinal BBM vesicles prepared from the intestines of normal and CF mice. Figure 6 demonstrates the effects of removal of luminal Cl^- on $J_{HCO_3^-}$. It is evident that the reduction of basal $J_{HCO_3^-}$ by removal of luminal Cl^- is similar in normal and CF mouse jejunum.

Since it is not entirely clear whether the percentage of HCO_3^- secretion which is dependent on luminal Cl^- is completely mediated by Cl^-–HCO_3^- exchange or whether changes in apical membrane potential or cell volume

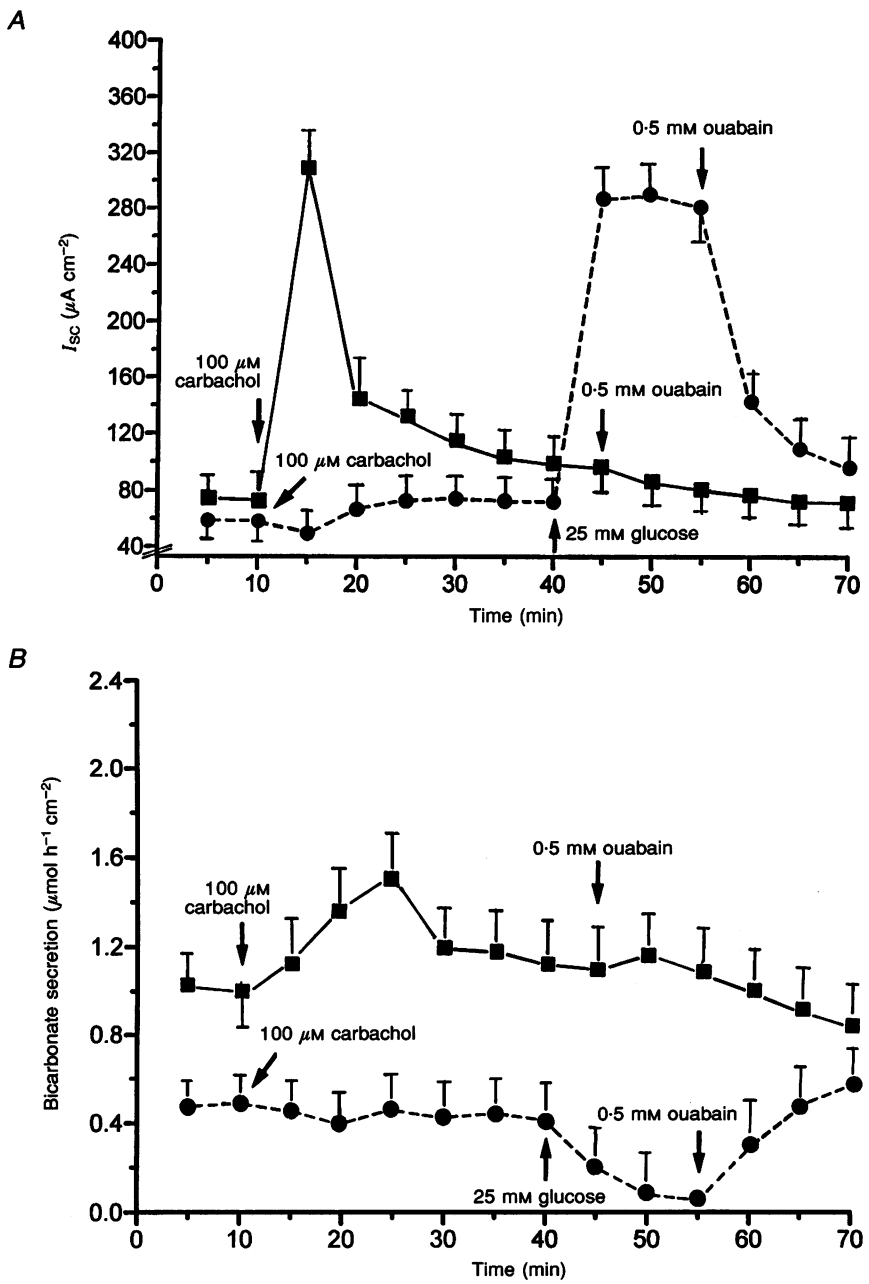

Figure 5. Time course of changes in I_{sc} and $J_{HCO_3^-}$ in normal (■) and CF mouse (●) jejunum in response to 100 μM carbachol and to 25 mM glucose in the CF mouse tissue

A, changes in I_{sc}. *B*, changes in $J_{HCO_3^-}$. $n = 4$–6.

influence HCO_3^- secretion as well, we also measured anion exchange activity in isolated small intestinal BBM vesicles In preliminary experiments, $^{36}Cl^-$ uptake into BBM vesicles, prepared from normal mouse small intestine, loaded with 50 mM HCO_3^- or 50 mM Cl^-, pH_i 7·5, was assessed and was quantitatively similar (18 ± 5 *vs.* 23 ± 4 nmol Cl^- (mg protein)$^{-1}$, respectively, at 10 s; $n = 4$, $P > 0{\cdot}05$). Comparison of anion exchange activity in BBM vesicles from normal and CF mouse intestine was then performed with Cl^--loaded vesicles. Figure 7 shows the time course of $^{36}Cl^-$ uptake into Cl^--loaded BBM vesicles of normal and CF mouse small intestine. Both the time course and peak values are not significantly different ($P > 0{\cdot}05$). Thus, one can conclude that the apical anion exchanger in the CF mouse small intestine is present and equally active when studied under the above conditions.

DISCUSSION

The study demonstrates that in all segments of the mouse small intestine, CFTR expression is mandatory for electrogenic HCO_3^- secretion. A large percentage of basal $J_{HCO_3^-}$ is dependent on CFTR channel expression and cAMP-, cGMP- and Ca^{2+}-dependent agonists stimulate HCO_3^- secretion exclusively via activation of the CFTR channel.

All tested cGMP-dependent agonists were also without any effect on small intestinal HCO_3^- secretion from CF mice. In a recent study (Guba *et al.* 1996) on HCO_3^- secretion in rat proximal duodenal mucosa *in vitro*, we observed that all tested cGMP-dependent agonists of duodenal anion secretion were non-additive to cAMP-dependent agonists, suggesting a common final pathway, most likely the CFTR channel. Nevertheless, the percentage of HCO_3^- secretion to I_{sc} stimulation was markedly higher for all cGMP-dependent agonists compared with that elicited by the cAMP-dependent agonists, making us doubt the then favoured theory that the cGMP-dependent agonists stimulate intestinal anion secretion via cross-activation of the protein kinase A (cAK) (Forte *et al.* 1992; Chao, de Sauvage, Dong, Wagner, Goeddel & Gardner, 1994). Indeed, we could show in a subsequent study that the effect of STa and cGMP analogues on intestinal anion secretion was markedly diminished in mice lacking the gene for the cGMP-dependent kinase II (cGKII), demonstrating that this kinase, and not protein kinase A, was the mediator for cGMP-dependent anion secretion (Pfeifer, Aszodi, Seidler, Ruth, Hofmann & Fässler, 1996). We therefore speculated that either cGMP-dependent agonists, via cGK II activation, stimulate additional HCO_3^- secretory mechanisms or, more likely, that the very different percentages of HCO_3^- to I_{sc} between cGMP- and cAMP-dependent agonists are due to the different distribution of the cGK II and the cAK along the crypt–villus axis (Markert *et al.* 1995) and a different anion availability in crypt *vs.* villus cells. The present study demonstrates that additional cGMP- but not cAMP-activatable HCO_3^- secretory pathways are not present in the small intestine and therefore rules out the first hypothesis.

Grubb has recently studied the changes in I_{sc} in normal and CF mouse jejunum upon forskolin and glucose stimulation (Grubb, 1995), and our results compare favourably with hers. She has not stripped the jejunum which probably explains her somewhat lower basal and peak values, as well as the TTX-sensitive oscillations of I_{sc} in normal mouse jejunum (which she explains by a tonic oscillatory release of neurotransmitters) and the larger difference in basal I_{sc} and PD between normal and CF tissue. On the basis of her finding that forskolin stimulates a small I_{sc} in the jejunum of normal but not CF mice incubated in bilaterally Cl^--free, HCO_3^--containing solution, which was reduced if HCO_3^- was removed bilaterally, she speculated that HCO_3^- may be secreted via CFTR channels in the absence of Cl^-. She pointed out that even if this were so, one would still not know if under physiological conditions the secreted HCO_3^-

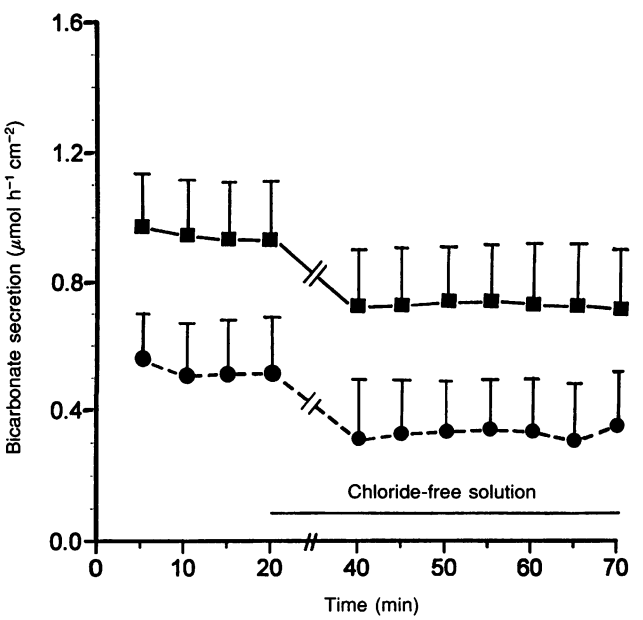

Figure 6. Changes in $J_{HCO_3^-}$ in normal (■) and CF (●) mouse jejunum after substitution of luminal Cl^- with gluconate

Removal of luminal Cl^- should inhibit HCO_3^- secretion by electroneutral Cl^-–HCO_3^- exchange. The bar indicates the period during which Cl^- was replaced by gluconate in equimolar concentration. $n = 5$.

passes through the CFTR channel, or if another transporter such as the anion exchanger is activated by the drop in $[Cl^-]_i$ secondary to CFTR channel activation. The present study only demonstrates that CFTR expression is necessary for electrogenic HCO_3^- secretion by any agonist, but does not imply that the exit of HCO_3^- ions into the intestinal lumen occurs via the CFTR channel. The CFTR channel is permeable to HCO_3^- (Gray *et al.* 1990; Poulsen *et al.* 1994), but theoretical considerations suggest that under the assumption of intracellular anion concentrations and an apical membrane potential found in more simple epithelia, the driving force for HCO_3^- exit in the presence of Cl^- should be fairly low (Kunzelmann, Gerlach, Fröbe & Greger, 1991). Other potential HCO_3^- exit pathways are another CFTR-dependent anion channel or the brush-border membrane anion exchanger. The first possibility is so far not supported by any experimental findings, but the second, although also not supported by experimental findings (Minhas *et al.* 1993; Guba *et al.* 1996), is theoretically very attractive and experimentally extremely difficult to rule out. We therefore thought it important to ascertain that no strong difference in small intestinal BBM anion exchange activity, possibly of a secondary nature, existed in the CF mice compared with normal mice. Indirect evidence that this is not the case came from the experiments which showed a reduction in $J_{HCO_3^-}$ upon removal of luminal Cl^-, consistent with $J_{HCO_3^-}$ secretion via Cl^-–HCO_3^- exchange, which was of similar magnitude in normal and CF mouse intestine. More direct assessment of BBM anion exchange was performed by measuring $^{36}Cl^-$ uptake into Cl^--loaded small intestinal BBM vesicles of normal and CF mice, and no significant differences in the time course and maximal uptake values were found. Since these flux studies are performed under maximal gradients for anion exchange, they give an indication of the number of active transporters in the membrane (which was the question), but of course they give no information about a potentially defective second-messenger regulation.

The addition of luminal glucose caused a strong increase in I_{sc}, due to activation of the Na^+–glucose cotransporter, indicating that no obvious villus atrophy was present. An interesting finding was that the addition of glucose caused a decrease in $J_{HCO_3^-}$ and sometimes even a temporary acidification of the luminal solution, and this was seen both in normal and CF mice intestine. One likely explanation for this finding is that the basal $J_{HCO_3^-}$ in the CF mouse, and a percentage of that in the normal mouse, small intestine is due to paracellular HCO_3^- permeation. On stimulation of nutrient absorption, the paracellular HCO_3^- serosa to mucosa flux is inhibited, possibly because of the increased PD negativity, and this decrease in paracellular HCO_3^- flux is masked during stimulation of the CFTR Cl^- conductance because of the increase in transcellular $J_{HCO_3^-}$. Our assumption that the major proportion of basal $J_{HCO_3^-}$ in the

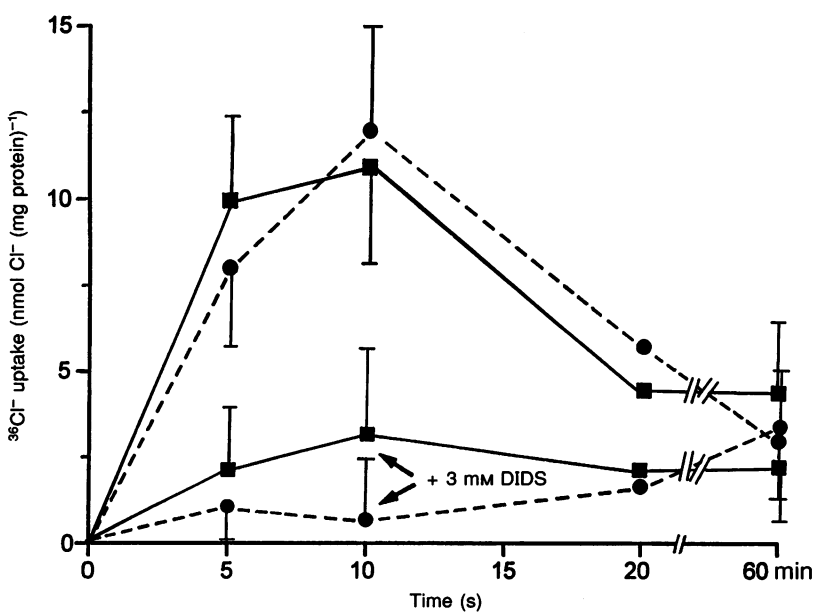

Figure 7. Time course of $^{36}Cl^-$ uptake into Cl^--loaded BBM vesicles prepared from normal (■) and CF mouse (●) small intestine

$[Cl^-]_i = 100$ mM, $[Cl^-]_o = 19$ mM after mixing 5 μl vesicle suspension with 45 μl uptake buffer. Vesicles were loaded with 100 mM KCl, 60 mM mannitol and 0·2 mM valinomycin, and the appropriate mixture of 40 mM Hepes–Tris–Mes to obtain a pH of 7·5. Extravesicular buffer consisted of 100 mM potassium gluconate, 10 mM $^{36}Cl^-$, 40 mM mannitol, 45 mM Hepes–Tris–Mes and 3 mM DIDS where indicated. Osmolarity was made exactly equal between intra- and extravesicular buffer. $n = 3$ (CF mice) and 5 (normal mice).

CF mouse intestine is paracellular is strengthened by the finding that in the CF, but not the normal mouse intestine, ouabain causes a rise in $J_{HCO_3^-}$. Ouabain has been previously reported to cause an increase in tight junction anion permeability (Bakker & Groot, 1984), and we have found an increase in the mannitol flux rates in rat duodenum upon ouabain treatment (authors' unpublished observations).

While not having any effect on $J_{HCO_3^-}$ in CF mouse small intestine, the addition of carbachol caused a biphasic change in I_{sc}, with an initial short decrease due to a drop in PD and a sustained increase due to a return of the PD to prestimulation values combined with a decrease in tissue resistance. Although presently not having an explanation for these findings, we mention them because of the obvious discrepancy in the literature regarding the existence of CFTR-independent Ca^{2+}-activated Cl^- channels in the intestine, as have been found in the airways (Anderson & Welsh, 1991) and which possibly also exist in certain intestinal cell lines (Vaandrager, Bajnath, Groot, Bot & de Jonge, 1991). A failure of cholinergic stimulation of intestinal anion secretion has been described in human CF intestinal epithelium (Taylor, Baxter, Hardcastle & Hardcastle, 1988; Berschneider *et al.* 1988) and by Clarke, Grubb, Yankaskas, Cotton, McKenzie & Boucher (1994) for the small and Cuthbert, MacVinish, Hickman, Ratcliff, Colledge & Evans (1994) for the large intestine of CF mice. Valverde, O'Brien, Sepulveda, Ratcliff, Evans & Colledge (1993), however, have found an absent VIP-induced volume decrease in small intestinal crypts isolated from the same CF mouse strain used in the present study, whereas that induced by carbachol was identical to the controls. The authors suggested that the preserved volume decrease upon stimulation with carbachol is most likely to be due to the existence of a separate apical Ca^{2+}-dependent, DIDS-inhibitable Cl^- channel in the small intestine, which is preserved in CF mice. In our study, the immediate, strong increase of I_{sc} elicited by carbachol in normal small intestine was absent in CF small intestine and therefore the predominant effect of carbachol on intestinal anion secretion is most likely to be due to an increase in the driving force for anion secretion via CFTR channels due to the activation of basolateral K^+ channels by carbachol (Dharmsathaphorn & Pandol, 1986). Whether the delayed and small increase in I_{sc} elicited by carbachol in CF small intestine in this study is related to a Ca^{2+}-activated anion transport pathway, possibly only existing in crypt cells, remains to be established.

In summary, the results demonstrate that the presence of the CFTR channel is necessary for agonist-induced stimulation of electrogenic HCO_3^- secretion in all segments of the small intestine. In the intestine, all three intracellular signal transduction pathways stimulate HCO_3^- secretion exclusively via activation of the CFTR channel.

Allen, A., Flemström, G., Garner, A. & Kivilaakso, E. (1993). Gastroduodenal mucosal protection. *Physiological Reviews* **73**, 823–857.

Anderson, M. P. & Welsh, M. J. (1991). Calcium and cAMP activate different chloride channels in the apical membrane of normal and cystic fibrosis epithelia. *Proceedings of the National Academy of Sciences of the USA* **88**, 6003–6007.

Bakker, R. & Groot, J. A. (1984). cAMP-mediated effects of ouabain and theophylline on paracellular ion selectivity. *American Journal of Physiology* **246**, G213–217.

Berschneider, H. M., Knowles, M. R., Azizkhan, R. G., Boucher, R. C., Tobey, N. A., Orlando, R. C. & Powell, D. W. (1988). Altered intestinal chloride transport in cystic fibrosis. *FASEB Journal* **2**, 2625–2629.

Brown, D. R., Parsons, A. M. & O'Grady, S. M. (1992). Substance P produces sodium and bicarbonate secretion in porcine jejunal mucosa through an action on enteric neurons. *Journal of Pharmacology and Experimental Therapeutics* **261**, 1206–1212.

Chao, A. C., de Sauvage, F. J., Dong, Y.-J., Wagner, J. A., Goeddel, D. V. & Gardner, P. (1994). Activation of intestinal CFTR Cl^- channel by heat-stable enterotoxin and guanylin via cAMP-dependent protein kinase. *EMBO Journal* **13**, 1065–1072.

Clarke, L. L., Grubb, B. R., Yankaskas, J. R., Cotton, C. U., McKenzie, A. & Boucher, R. C. (1994). Relationship of a non-cystic fibrosis transmembrane conductance regulator-mediated chloride conductance in organ-level disease in CFTR (−/−) mice. *Proceedings of the National Academy of Sciences of the USA* **91**, 479–483.

Cuthbert, A. W., MacVinish, L. J., Hickman, M. E., Radcliff, R., Colledge, W. H. & Evans, M. J. (1994). Ion-transporting activity in the murine colonic epithelium of normal animals and animals with cystic fibrosis. *Pflügers Archiv* **428**, 508–515.

Dagher, P. C., Rho, J. I. & Charney, A. N. (1993). Mechanism of bicarbonate secretion in rat colon. *Comparative Physiology* **105**, 43–48.

Dahlquist, A. (1964). A method for assay of intestinal disaccharidases. *Analytical Biochemistry* **7**, 18–25.

Davis, G. R., Morawski, S. G., Santa Ana, C. A. & Fordtran, J. S. (1983). Evaluation of chloride/bicarbonate exchange in the human colon *in vivo*. *Journal of Clinical Investigation* **71**, 201–207.

Dharmsathaphorn, K. & Pandol, S. J. (1986). Mechanisms of chloride secretion induced by carbachol in a colonic epithelial cell line. *Journal of Clinical Investigation* **77**, 348–354.

Dietz, J. & Field, M. (1973). Ion transport in rabbit ileal mucosa. IV. Bicarbonate secretion. *American Journal of Physiology* **225**, G859–861.

Flemström, G. & Garner, A. (1982). Gastroduodenal HCO_3^- transport: characteristics and proposed role in acidity regulation and mucosal protection. *American Journal of Physiology* **242**, G183–193.

Flemström, G., Garner, A., Nylander, O., Hurst, B. C. & Heylings, J. R. (1982). Surface epithelial HCO_3^- transport by mammalian duodenum *in vivo*. *American Journal of Physiology* **243**, G348–358.

Flemström, G., Heylings, J. R. & Garner, A. (1982). Gastric and duodenal HCO_3^- transport *in vitro*: effects of hormones and local transmitters. *American Journal of Physiology* **242**, G100–110.

Forbush, B. (1983). Assay of Na/K ATPase in plasma membrane preparations: increasing the permeability of membrane vesicles using sodium dodecyl sulphate buffered with bovine serum albumine. *Analytical Biochemistry* **128**, 159–163.

FORTE, L. R., THORNE, P. K., EBER, S. L., KRAUSE, W. J., FREEMAN, R. H., FRANCIS, S. H. & CORBIN, J. C. (1992). Stimulation of intestinal Cl^- secretion by heat-stable enterotoxin: activation of cAMP-dependent protein kinase by cGMP. *American Journal of Physiology* **263**, C607–615.

GRAY, M. A., POLLARD, C. E., HARRIS, A., COLEMAN, L., GREENWELL, J. R. & ARGENT, B. R. (1990). Anion selectivity and block of the small-conductance chloride channel on pancreatic duct cells. *American Journal of Physiology* **259**, C752–761.

GRUBB, B. R. (1995). Ion transport across the jejunum in normal and cystic fibrosis mice. *American Journal of Physiology* **268**, G505–513.

GUBA, M., KUHN, M., FORSSMANN, W. G., CLASSEN, M., GREGOR, M. & SEIDLER, U. (1996). Guanylin strongly stimulates rat duodenal HCO_3^- secretion: proposed mechanism and comparison with other secretagogues. *Gastroenterology* **111**, 1558–1568.

HANNA, S., MIRCHEFF, A. K. & WRIGHT, E. M. (1979). Alkaline phosphatase of basal lateral and brush border plasma membranes from intestinal epithelium. *Journal of Supramolecular Structure* **11**, 451–466.

HEYLINGS, J. R. & FELDMAN, M. (1988). Basal and PG E_2-stimulated duodenal bicarbonate secretion in the rat *in vivo*. *American Journal of Physiology* **255**, G470–475.

HUBEL, K. A. (1967). Bicarbonate secretion in rat ileum and its dependence on intraluminal chloride. *American Journal of Physiology* **213**, 1409–1413.

ISENBERG, J. I., LJUNGSTRÖM, M., SÄFSTEN, B. & FLEMSTRÖM, G. (1993). Acid/base transport by proximal duodenal enterocytes: evidence for Na^+/H^+ and Cl^-/HCO_3^-exchange and $NaHCO_3^-$ cotransport. *American Journal of Physiology* **265**, G677–685.

KNICKELBEIN, R., ARONSON, P. S., ATHERTON, W. & DOBBINS, J. W. (1983). Sodium and chloride transport across rabbit ileal brush border. I. Evidence for Na–H exchange. *American Journal of Physiology* **245**, G504–510.

KNICKELBEIN, R., ARONSON, P. S., ATHERTON, W. & DOBBINS, J. W. (1985). Sodium and chloride transport across rabbit ileal brush border. II. Evidence for $Cl–HCO_3$ exchange. *American Journal of Physiology* **249**, G236–245.

KUNZELMANN, K., GERLACH, L., FRÖBE, U. & GREGER, R. (1991). Bicarbonate permeability of epithelial chloride channels. *Pflügers Archiv* **417**, 616–621.

MARKERT, T., VAANDRAGER, A. B., GAMBARYAN, S., PÖHLER, D., HÄUSLER, C., WALTER, U., DE JONGE, H. R., JARCHAU, T. & LOHMANN, S. M. (1995). Endogenous expression of type II cGMP-dependent protein kinase mRNA and protein in rat intestine: implications for CFTR regulation. *Journal of Clinical Investigation* **96**, 822–830.

MINHAS, B. S., SULLIVAN, S. K. & FIELD, M. (1993). Bicarbonate secretion in rabbit ileum: electrogenicity, ion dependence, and effects of cyclic nucleotides. *Gastroenterology* **105**, 1617–1629.

NOVAK, I. & GREGER, R. (1988). Properties of the luminal membrane of isolated perfused rat pancreatic ducts: Effect of cyclic AMP and blockers of chloride transport. *Pflügers Archiv* **411**, 546–553.

PETERSON, G. L. (1983). Determination of total protein. *Methods in Enzymology* **91**, 95–119.

PFEIFER, A., ASZODI, A., SEIDLER, U., RUTH, P., HOFMANN, F. & FÄSSLER, R. (1996). Intestinal secretory defects and dwarfism in mice lacking cGMP-dependent protein kinase II. *Science* **274**, 2082–2087.

POULSEN, J. H., FISCHER, H., ILLEK, B. & MACHEN, T. E. (1994). Bicarbonate conductance and pH-regulatory capability of cystic fibrosis transmembrane conductance regulator. *Proceedings of the National Academy of Sciences of the USA* **91**, 5340–5344.

RATCLIFF, R., EVANS, M. J., CUTHBERT, A. W., MACVINISH, L. J., FOSTER, D., ANDERSON, J. R. & COLLEDGE, W. H. (1993). Production of a severe cystic fibrosis mutation in mice by gene targeting. *Nature Genetics* **4**, 35–41.

STRONG, T. V., BOEHM, K. & COLLINS, F. S. (1994). Localization of cystic fibrosis transmembrane conductance regulator mRNA in the human gastrointestinal tract by *in situ* hybridization. *Journal of Clinical Investigation* **93**, 347–354.

TAYLOR, C. J., BAXTER, P. S., HARDCASTLE, J. & HARDCASTLE, P. T. (1988). Failure to induce secretion in jejunal biopsies from children with cystic fibrosis. *Gut* **29**, 957–962.

TREZISE, A. E. O. & BUCHWALD M. (1991). *In vivo* cell-specific expression of the cystic fibrosis transmembrane regulator. *Nature* **353**, 434–437.

TURNBERG, L. A., BIEBERDORF, F. A., MORAWSKI, S. G. & FORDTRAN, J. S. (1970). Interrelationships of chloride, bicarbonate, sodium and hydrogen transport in the human ileum. *Journal of Clinical Investigation* **49**, 557–567.

VAANDRAGER, A. B, BAJNATH, R., GROOT, J. A., BOT, A. G. M & DE JONGE, H. R. (1991). Ca^{2+} and cAMP activate different chloride efflux pathways in HT-29.cl19A colonic epithelial cell line. *American Journal of Physiology* **261**, G958–965.

VALVERDE, M. A., O'BRIEN, J. A., SEPULVEDA, F. V., RATCLIFF, R., EVANS, M. J. & COLLEDGE, W. H. (1993). Inactivation of the murine *cftr* gene abolishes cAMP-mediated but not Ca^{2+}-mediated secretagogue-induced volume decrease in small intestinal crypts. *Pflügers Archiv* **425**, 434–438.

YAO, B., HOGAN, D. L., BUKHAVE, K., KOSS, M. A. & ISENBERG, J. I. (1993). Bicarbonate transport by rabbit duodenum *in vitro*: Effect of vasoactive intestinal polypeptide, prostaglandin E_2, and cyclic adenosine monophosphate. *Gastroenterology* **104**, 732–740.

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