Cystic Fibrosis Transmembrane Conductance Regulator Regulates Luminal Cl⁻/HCO₃⁻ Exchange in Mouse Submandibular and Pancreatic Ducts*

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We have demonstrated previously the regulation of Cl⁻/HCO₃⁻ exchange activity by the cystic fibrosis transmembrane conductance regulator (CFTR) in model systems of cells stably or transiently transfected with CFTR (Lee, M. G., Wigley, W. C., Zeng, W., Noel, L. E., Marino, C. R., Thomas, P. J., and Muallem, S. (1999) J. Biol. Chem. 274, 3414–3421). In the present work we examine the significance of this regulation in cells naturally expressing CFTR. These include the human colonic T84 cell line and the mouse submandibular gland and pancreatic ducts, tissues that express high levels of CFTR in the luminal membrane. As in heterologous expression systems, stimulation of T84 cells with forskolin increased the Cl⁻/HCO₃⁻ exchange activity independently of CFTR Cl⁻ channel activity. Freshly isolated submandibular gland ducts from wild type mice showed variable Cl⁻/HCO₃⁻ exchange activity. Measurement of [Cl⁻]l revealed that this was largely the result of variable steady-state [Cl⁻]l. Membrane depolarization with 5 mM Ba²⁺ or 100 mM K⁺ increased and stabilized [Cl⁻]l. Under depolarized conditions wild type and ΔF/ΔF mice had comparable basal Cl⁻/HCO₃⁻ exchange activity. Notably, stimulation with forskolin increased Cl⁻/HCO₃⁻ exchange activity in submandibular gland ducts from wild type but not ΔF/ΔF mice. Microperfusion of the main pancreatic duct showed Cl⁻/HCO₃⁻ exchange activity in both the basolateral and luminal membranes. Stimulation of ducts from wild type animals with forskolin had no effect on basolateral but markedly stimulated luminal Cl⁻/HCO₃⁻ exchange activity. By contrast, forskolin had no effect on either basolateral or luminal Cl⁻/HCO₃⁻ exchange activity of ducts from ΔF/ΔF animals. We conclude that CFTR regulates luminal Cl⁻/HCO₃⁻ exchange activity in CFTR-expressing cells, and we discuss the possible physiological significance of these findings regarding cystic fibrosis.

HCO₃⁻ secretion is a primary function of many CFTR¹-expressing cells (1–3). Most of the HCO₃⁻ is secreted by duct or duct-like cells to the lumen and thus requires transudctal HCO₃⁻ transport. Little is known about the pathways mediating HCO₃⁻ entry in the basolateral membrane (BLM). The best studies available to date are in the pancreatic duct, in which Case and co-workers (4–6) provided strong evidence that HCO₃⁻ influx is mediated largely by a BLM Na⁺/HCO₃⁻ co-transport. HCO₃⁻ efflux across the luminal membrane (LM) and its regulation are equally poorly understood. Most models assume that the electroneutral component of HCO₃⁻ secretion is mediated by a luminal Cl⁻/HCO₃⁻ exchanger (AE, anion exchanger) (1–3, 6). This function is also believed to mediate part of Cl⁻ absorption by the duct. Cl⁻ is supplied to the duct lumen in the plasma-like primary fluid secreted by acinar cells (1–3, 7). The pathophysiology of cystic fibrosis indicates that CFTR plays a critical, but poorly defined, role in HCO₃⁻ secretion and Cl⁻ absorption. In tissues such as the salivary glands, in which acinar cells secrete the bulk of the fluid, CFTR is assumed to mediate the electrogenic part of Cl⁻ absorption (2). The same role is attributed to CFTR in sweat glands (7) and intestinal epithelia (8, 9). Recent work suggests that airway epithelia absorb Na⁺ and Cl⁻ to produce a hypotonic airway surface fluid (Ref. 10 and references within, but see Ref. 11 and references within). Because Na⁺ and Cl⁻ concentrations in airway surface liquid produced by cystic fibrosis airway epithelium are isotonic (10, 11), CFTR may mediate electrogenic Cl⁻ absorption in airway epithelia (12, 13). In glands like the pancreas, fluid secretion by acinar cells is limited, and the duct secretes the bulk of the fluid in pancreatic juice (1). In this type of gland the limited supply of Cl⁻ secreted by acinar cells led to the proposal that CFTR mediates Cl⁻ secretion to the lumen of duct cells to fuel the Cl⁻/HCO₃⁻ exchanger (1). However, a recent work showed that agonist- and cAMP-stimulated HCO₃⁻ secretion in guinea pig pancreatic duct is independent of luminal Cl⁻ (6). Hence, the role of CFTR in ion transport by these tissues remains obscure.

To date direct evidence in support of the two models is meager indeed. Localization of CFTR in the luminal membrane of all CFTR-expressing epithelia is well documented (14–16). Cl⁻/HCO₃⁻ exchange activity was found in the BLM and LM of pancreatic (17) and submandibular gland (SMG) ducts (18). In SMG ducts the AE isoform 2 (AE2) was localized in the BLM (19). The isoform(s) expressed in the LM is not known.

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¹ The abbreviations used are: CFTR, cystic fibrosis transmembrane conductance regulator; BLM, basolateral membrane; LM, luminal membrane; AE, Cl⁻/HCO₃⁻ (anion) exchanger; SMG, submandibular gland; WT, wild type; ΔF, deletion mutant of Phe-508 from WT CFTR; PSA, pancreatic solution A; BCECF-AM, 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester; MQAE, 1-naphthylmethyl-6-methoxyquinolinium bromide; DIDS, 4,4′-diisothiocyanato-stilbene-2,2′-disulfonate.
A relationship between HCO₃⁻ secretion and CFTR was documented in two cell lines and intestinal epithelia. In a human airway epithelial cell line CFTR-dependent HCO₃⁻ conductance (20, 21) was proposed to be mediated by CFTR itself. By contrast, similar studies in a human pancreatic duct cell line concluded that electrogenic Cl⁻ and HCO₃⁻ secretions are mediated by independent proteins (22). In duodenal epithelium basal and acid-stimulated HCO₃⁻ secretions were reduced or absent in CFTR −/− mice (23). Surprisingly, in a recent study Seidler et al. (24) showed that all forms of HCO₃⁻ secretion stimulated by agonists or agents that elevate cAMP, cGMP, and, in particular, [Ca²⁺]i, were impaired in the intestinal epithelia of CFTR −/− mice. These studies suggest the likely involvement of CFTR in the electrogenic component of HCO₃⁻ secretion, which is particularly prominent in the intestine (25, 26). However, a large fraction of HCO₃⁻ secretion in tissues such as salivary glands (2) and the rat and mouse pancreas (1) is mediated by an electroneutral HCO₃⁻ transport mechanism. The role of CFTR in this critical component of HCO₃⁻ secretion is unknown.

The intimate relationship between CFTR expression and HCO₃⁻ secretion seen in intestinal (23, 24) and airway epithelia (27) raises the question of whether and how CFTR modulates HCO₃⁻ secretion in other CFTR-expressing tissues. In addition to its possible function as a regulator of a HCO₃⁻ conductive channel, CFTR may also regulate luminal Cl⁻/HCO₃⁻ exchange activity. In a recent study (28) we used cells stably transfected with CFTR and transient transfection of WT CFTR and several CFTR mutants to demonstrate regulation of AE activity by CFTR. To evaluate the physiological relevance of these findings, in the present work we report the regulation of the luminal AE activity by CFTR in the mouse SMG and pancreatic ducts.

The SMG and pancreatic ducts were selected as model systems for several reasons. The fraction of electroneutral HCO₃⁻ secretion in these tissues is relatively high (1, 2). Among all CFTR-expressing tissues, the mechanism of fluid and electrolyte secretion is understood best in the SMG (2). The SMG and pancreatic ducts express high levels of CFTR (15), and the extent of pHi modifications of our published procedure (16). Mice were sacrificed by cervical dislocation (ATCC CCL 248, Rockville, MD) and maintained in a 1:1 mixture of Ham’s F-12 medium and Dulbecco’s modified Eagle’s medium supplemented with 5% fetal calf serum. The cells were plated on a sterile 22 × 40-mm coverslip at a density of 2.5 × 10⁶ cells/cm² for intracellular pH measurements.

**Animals and Preparation of Ducts** — A cystic fibrosis mouse model in which the ΔF508 mutation was introduced in the mouse CFTR by gene targeting in ES cells (31) was obtained from Dr. Kirk R. Thomas (Eccles Institute of Human Genetics, HHMI, University of Utah School of Medicine, Salt Lake City). The mice were maintained on a standard diet, and genotyping was carried out on day 14 postpartum as described previously (16).

Duct fragments from the mouse SMG were prepared by a slight modification of our published procedure (16). Mice were sacrificed by exposure to a methoxyflurane-saturated atmosphere and subsequent cervical dislocation, and the SMGs were removed to a cold pancreatic solution A (PSA). The composition of PSA was (in mM) 140 NaCl, 5 KCl, 1 MgCl₂, 1 CaCl₂, 10 Heps (pH 7.4 with NaOH), 10 glucose, 10 pyruvate, 0.1% bovine serum albumin, and 0.02% soybean trypsin inhibitor. Each gland was cleaned by injection of 5 ml of PSA and minced. The mixture was transferred to 8 ml of PSA containing 2.5 mg of collagenase (CLS4, 254 units/mg; Worthington Biochemical) and digested for 8–10 min at 37 °C. The dissociated cells were then washed twice with PSA, resuspended in 2 ml of PSA, and kept on ice until use.

Microperfusion experiments were performed with microdissected pancreatic ducts from WT and ΔF508 mice. The procedure for preparation and perfusion of the main pancreatic duct was identical to that used for perfusion of the rat pancreatic duct (17). The ducts were dissected in PSA, cannulated, and perfused through the lumen and the bath with solution A. After completion of BCEE CF loading the ducts were perfused with HCO₃⁻-buffered solution B for at least 10 min prior to manipulation of Cl⁻ gradients.

**Intracellular pH (pHi) Measurements** — The procedure of pHi measurement in T84 cells was identical to that described in detail in our recent work (28). In the case of SMG cells, the dissociated cells were loaded with BCEE CF by a 10-min incubation at room temperature in PSA containing 1 μM BCEE CF-AM. The cells were then washed with PSA and plated on a polylisine-coated coverslip that was assembled into a perfusion chamber. The chamber was placed on an inverted microscope, and intralobular ducts were identified by morphology. The BCECF fluorescence of 10–16 cells of a duct fragment was recorded at excitation wavelengths of 440 and 490 nm. Fluorescence ratios of 490/440 were calibrated using the procedures described previously (28). In the case of the perfused pancreatic duct BCEE CF loading was accomplished by including 2.5 μM BCEE CF-AM in the luminal perfusate for 10 min.

Changes in Cl⁻/HCO₃⁻ exchange activity were estimated from the integral of pH changes (T84 cells and pancreatic ducts) or from the extent of pHi changes (SMG ducts). Initial rates of pH changes were obtained from the first derivative of the traces using a single exponential fit. The extent of pH changes was estimated by averaging the pH changes measured as a result of Cl⁻ removal and addition. All results are given as mean ± S.E. of the indicated number of experiments.

**Intercellular Cl⁻ Measurement** — [Cl⁻]i was measured with the aid of the Cl⁻-sensitive dye MQAE using the procedure described before for 6-methoxy-N-(3-sulfopropyl)quinolinium (SPQ; 18) with minor modifications. SMG cells were suspended in PSA containing 10 mM MQAE and incubated for 20 min at room temperature and 40 min at 0 °C before plating on coverslips. About 2 min after plating, unattached cells and external MQAE were washed by starting the perfusion with solution A. MQAE fluorescence was measured at an excitation wavelength of 360 nm with the dichroic mirror and emission cut-off filter set normally used to monitor Fura-2 fluorescence. At the end of each experiment a two-point calibration procedure was performed. To obtain the maximal fluorescence the cells were perfused with high K⁺, Cl⁻-free solution containing 5 μM nigericin and 10 μM tributyltin cyanide. Incubation in a Cl⁻-free solution without ionophores did not result in comparable changes. The Cl⁻-free solution containing the cells were then exposed to a solution containing 127 mM KSCN. Significant dye leak, in particular after exposure to tributyltin cyanide, precluded a more extensive in vivo calibration. A Stern-Volmer constant of 12.4 μM⁻¹ reported for rabbit SMG ducts (34) was used to calculate [Cl⁻]i.

The results of multiple experiments with each cell type and under the
results

Regulation of AE Activity in T84 Cells—We have described previously the regulation of AE activity by CFTR in cells stably or transiently transfected with CFTR (28). The purpose of the present work was to determine whether such regulation exists in a cell line and in native cells naturally expressing CFTR. The first set of experiments was performed with the human colonic cell line T84, which can serve as a suitable model system in future studies. This cell line has been used in the past in several studies as a model system to characterize natively expressed CFTR (32, 33).

Fig. 1 shows representative experiments, and Fig. 2 summarizes the results under each experimental condition. DIDS-sensitive, Cl⁻- and HCO₃⁻-dependent changes in pH, indicate the expression of relatively modest AE activity in T84 cells. Stimulation of the cells with 5 μM forskolin caused a reproducible reduction in pH. This reduction in pH was less pronounced than that observed in NIH 3T3 and HEK 293 cells expressing high levels of CFTR. Removal and addition of Cl⁻ to the incubation medium showed that forskolin increased the rate of AE activity by about 2.2-fold or 0.051 pH unit/min. As was found in NIH 3T3 and HEK 293 cells expressing CFTR (28), the AE activity stimulated by forskolin was not affected by inhibition of CFTR-mediated Cl⁻ current with 0.1 mM N-phenylanthranilic acid (DPC) (panel a), or 0.1 mM glibenclamide (Glib), and then 0.5 mM DIDS (panel b), or high K⁺-containing medium (panel c) before and during removal and addition of Cl⁻. Upper deflection in all traces indicates an increase in pH. The results of multiple experiments are summarized in Fig. 2.

different conditions were analyzed using paired or nonpaired Student’s t test, as appropriate.

Figs. 1c and 2, T84 cells were depolarized by raising the external K⁺ concentration from 5 to 100 mM. This had a minor effect on pH. Membrane depolarization nearly doubled the initial rate of HCO₃⁻ influx observed upon Cl⁻ removal (Figs. 1c and 2, first and second bars from left). Stimulation with forskolin of cells bathed in high K⁺ medium increased AE activity by about 1.9-fold (compare third and fourth bars of Fig. 2), similar to the stimulation found in the presence of 5 mM external K⁺ (first and second bars in Fig. 2). However, in the presence of 100 mM K⁺, forskolin stimulation increased the absolute rate of HCO₃⁻ influx by 0.082 pH unit/min, which was approximately 1.6-fold higher than that found in normal K⁺ medium (Fig. 2, inset). The simplest interpretation of these results is that membrane depolarization increased the steady-state level of intracellular Cl⁻ (see below). If the internal Cl⁻ site of the AE was not saturated with Cl⁻ present in the cells under normal conditions, the increased [Cl⁻], caused by membrane depolarization will increase the rate of Cl⁻/HCO₃⁻ exchange. Another contributing factor can be reduction in a potential HCO₃⁻ permeability under depolarized conditions. An additional implication of the findings in Fig. 1c is that most of the HCO₃⁻ fluxes induced by changes in transcellular Cl⁻ gradients are caused by the electroneutral AE activity.

AE Activity in the SMG of WT and ΔF/ΔF Mice—A critical aspect of the regulation of AE activity by CFTR is to determine whether it occurs in native CFTR-expressing cells. We elected to study the relationship between the two proteins in the mouse SMG and pancreatic ducts because of the availability of the ΔF/ΔF mouse strain. The rat and mouse SMG and pancreatic ducts express a high level of CFTR protein in the luminal membrane (16) and Cl⁻/HCO₃⁻ exchange activity in the basolateral and luminal membranes (17, 18). However, a previous study in the perfused main duct of the mouse SMG suggested very low, if any, Cl⁻/HCO₃⁻ exchange activity in either membrane of this duct (30). We reevaluated these findings by measuring Cl⁻/HCO₃⁻ exchange activity in the intralobular duct of the mouse SMG. As illustrated in Fig. 3, a and b, we found high variability in Cl⁻/HCO₃⁻ exchange activity in isolated SMG ducts. The traces in Fig. 3, a and b, represent the range of high and low AE activity in SMD ducts from WT mice, respectively. Fig. 3c shows AE activity in a SMG duct fragment from a ΔF/ΔF mouse. The AE activity of ducts from mutant mice was less variable, as reflected in the averaged results (Fig. 3d). In a
**AE and CFTR in Secretory Ducts**

**Fig. 3. AE activity in the SMG duct of WT and ΔF/ΔF mice.** Ducts were isolated from the SMG of WT (panels a and b) or ΔF/ΔF (panel c) mice and used to measure pH in ducts from WT animals. The same protocol was used to evaluate AE activity in SMG ducts from ΔF/ΔF mice. Because of the regulation of AE activity by CFTR demonstrated before (28), we expected to find the same or lower AE activity in the SMG duct of ΔF/ΔF mice. Surprisingly, AE activity in ducts isolated from SMG of ΔF/ΔF mice was significantly higher than in ducts isolated from the SMG of WT mice (Fig. 3, c and d).

**Table I**

| [Cl<sup>−</sup>]<sub>i</sub> in control and depolarized submandibular duct cells | [Cl<sup>−</sup>]<sub>i</sub> | Δ[Cl<sup>−</sup>]<sub>i</sub> |
|---|---|---|
| mm | mm | |
| Control (n = 17) | 23.7 ± 1.9 | |
| Forskolin (n = 4) | 25.3 ± 2.1 | 1.6 |
| High K<sup>+</sup> (n = 7) | 28.9 ± 1.2* | 5.2 |
| High K<sup>+</sup> + forskolin (n = 5) | 28.6 ± 1.1* | 4.9 |
| Ba<sup>2+</sup> (n = 4) | 28.0 ± 0.7* | 4.3 |

* p < 0.05 relative to control.

Only did membrane depolarization increase [Cl<sup>−</sup>]<sub>i</sub>, but it also reduced the variability of [Cl<sup>−</sup>]<sub>i</sub> in unstimulated cells (Table I), which facilitated evaluation of Cl<sup>−</sup>/HCO<sub>3</sub> exchange activity in cells from WT and ΔF/ΔF mice. Hence, in most subsequent experiments Cl<sup>−</sup>/HCO<sub>3</sub> exchange activity was measured under depolarized conditions.

**AE Activity under Depolarized Conditions**—The effect of membrane depolarization with Ba<sup>2+</sup> on Cl<sup>−</sup>/HCO<sub>3</sub> exchange is illustrated in Fig. 4. In SMG ducts from WT mice, inhibition of K<sup>+</sup> conductance is expected to depolarize the membrane and reduce Cl<sup>−</sup> efflux, increasing and stabilizing steady-state [Cl<sup>−</sup>]<sub>i</sub>. As a consequence, the maximal Cl<sup>−</sup>/HCO<sub>3</sub> exchange activity of these cells could be measured. In SMG ducts from ΔF/ΔF mice membrane depolarization is expected to have smaller, if any, effect on the Cl<sup>−</sup>-dependent changes on pH.<sup>2</sup> Importantly, membrane depolarization minimizes the contribution of all Cl<sup>−</sup> channels expressed in these cells (39) to allow a better comparison between the electroneutral Cl<sup>−</sup>/HCO<sub>3</sub> exchange activity of ducts from WT and ΔF/ΔF mice. The traces of an individual experiment (Fig. 4, a and b) and the averages obtained from multiple experiments (Fig. 4c) show that this was indeed the case.

In these experiments Ba-Hepes (0.5 m, pH 7.4) was added to HCO<sub>3</sub>-buffered solutions in which Cl<sup>−</sup> was replaced by gluconate. These solutions were clear for about 30 min, after which precipitates, probably of Ba-gluconate, started to form. At this time old solutions were replaced with fresh Ba<sup>2+</sup>-containing, Cl<sup>−</sup>-free solutions. Exposing SMG ducts bathed in a HCO<sub>3</sub>-buffered medium to 5 mM Ba<sup>2+</sup> caused a small and reproducible reduction in pH<sub>i</sub>, the cause of which was not investigated in the present work. Membrane depolarization with Ba<sup>2+</sup> increased the apparent AE activity by more than 2-fold in SMG ducts from WT mice. By contrast, Ba<sup>2+</sup> had a small, statistically insignificant effect on AE activity of SMG ducts from ΔF/ΔF mice. Furthermore, in the presence of Ba<sup>2+</sup>, the AE activity of SMG duct from WT mice tended to be higher than that of ducts from ΔF/ΔF mice, although it did not reach statistical significance (p = 0.083). Finally, the Cl<sup>−</sup>-dependent changes in pH<sub>i</sub> were blocked completely by DIDS (Fig. 4, a and b).

**Regulation of AE Activity by Forskolin**—The effect of forskolin stimulation on AE activity of SMG duct from WT and ΔF/ΔF mice is shown in Fig. 5. Fold stimulation was determined from the extent of pH<sub>i</sub> changes caused by Cl<sup>−</sup>-removal and addition before and after forskolin stimulation of the same duct fragments. Even in the absence of Ba<sup>2+</sup> stimulation of SMG duct from WT mice with forskolin increased AE activity by about 1.7-fold. By contrast, and as expected, forskolin had no effect on the AE activity of ducts isolated from the SMG of ΔF/ΔF mice. We noticed that multiple removals and additions of Cl<sup>−</sup> to the incubation medium resulted in a slightly reduced AE activity in each successive round. Thus, the small, frequently observed...
reduction in AE activity in forskolin-stimulated ducts from ΔF/ΔF mice is probably the result of this artifact. Because in most experiments a control test preceded an experimental test, the effect of forskolin on AE activity in SMG duct from WT mice is probably underestimated. Nevertheless, it was sufficiently large to be highly statistically significant (Fig. 5c).

To determine the actual stimulation of AE by CFTR in SMG duct we measured the effect of forskolin in ducts treated with 5 mM Ba²⁺. Fig. 6 shows that forskolin stimulation of SMG ducts from WT mice, but not from ΔF/ΔF mice, incubated in Ba²⁺-containing solutions increased AE activity. In SMG ducts from WT mice, forskolin increased pH₂ by 0.06 pH unit above that measured in the same unstimulated ducts, which consists of an approximately 1.3-fold stimulation. However, when pH₂ changes caused by AE activity are compared in forskolin-stimulated ducts from WT and ΔF/ΔF mice, pH₂ changes in WT ducts are higher by about 0.12 pH unit, which is a 1.9-fold above that measured in ducts from ΔF/ΔF mice. This stimulation is comparable to that found in the absence of Ba²⁺ as illustrated in Fig. 5.

To avoid the possibility of a nonspecific effect of Ba²⁺, we tested the effect of high K⁺ on AE activity in SMG ducts from WT mice. Fig. 7 shows that depolarizing the membrane potential with 100 mM K⁺ was as effective as 5 mM Ba²⁺ in unmasking the maximal AE activity. As expected, the CI⁻-dependent pH₂ changes were inhibited by DIDS (Fig. 7a). Stimulation of the ducts with forskolin increased the AE activity of these ducts. In these experiments we compared the AE activity measured in the first exposure of all ducts to Cl⁻-free medium in the presence of 100 mM K⁺ or 5 mM Ba²⁺ were similar.

Membrane-specific Regulation of CI⁻/HCO₃⁻ Exchange by CFTR in Pancreatic Ducts—CFTR-expressing cells are likely to express more than one anion exchanger, the housekeeping AE2 in the basolateral membrane and as yet unidentified AE isoform or other exchanger protein involved in transcellular HCO₃⁻ transport in the luminal membrane. Indeed, AE activity was measured previously in both membranes of the SMG (18) and pancreatic ducts (17). To extend our findings to another native CFTR-expressing tissue and determine the membrane localization of the AE activity regulated by CFTR, we measured luminal and basolateral AE activity in the micropерfused pancreatic ducts of WT and ΔF/ΔF mice. Fig. 8 summarizes the results of multiple experiments. It was satisfying to find that stimulation with forskolin exclusively increased the activity of the luminal AE without affecting the basolateral AE in pancreatic ducts from WT mice. Furthermore, such regulation was absent in ducts from ΔF/ΔF animals. In the first protocol we measured AE activity under polarized conditions (Fig. 8a). As we reported before for the rat duct, luminal AE activity was higher than basolateral AE activity in the mouse pancreatic...
duct (compare the rate and extent of pH changes caused by the Cl− removal from the bath and lumen in panels a and b before forskolin stimulation). Stimulation with forskolin caused the typical initial acidification, had no effect on basolateral AE activity, and prominently increased luminal AE activity in ducts from WT mice. Forskolin had no effect on luminal or basolateral AE activity of ducts from ΔF/ΔF mice (data not shown).

To obtain a better estimate of the AE activity in each membrane and under resting and stimulated conditions, we measured separately the basolateral and luminal AE activity under depolarized conditions. The left part of Fig. 8b shows the higher rate (and extent) of pH changes caused by Cl− removal and addition to the lumen (and thus the higher AE activity) of pancreatic duct from WT mice. The right part of the trace shows that stimulation with forskolin had no effect on the basolateral AE activity but increased the initial rate (and extent) of pH changes because of removal of luminal [Cl−]out in the same duct. Both activities were inhibited completely by 0.5 mM DIDS. By contrast, forskolin had no effect on basolateral and luminal AE activity of ducts from ΔF/ΔF mice (data not shown). Hence, we can conclude that CFTR regulates the luminal but not the basolateral AE activity.

**DISCUSSION**

In the present work we used the SMG and pancreatic ducts of WT and ΔF/ΔF mice to study regulation of Cl−/HCO3− exchange activity by CFTR. Along with our experience with these cells, the SMG and pancreatic ducts offer other advantages as model systems. Both ducts express high levels of CFTR (16) and basolateral and luminal AE activity (17, 18). In addition, the SMG offers the availability of several experimental preparations from microperfused ducts (2, 18) to isolated single cells (2, 16); and most importantly, among all CFTR-expressing tissues, the mechanisms of fluid and electrolyte transport are understood best in the SMG (2).

In agreement with our previous work in the rat SMG duct (18), in the present work we were able to demonstrate AE activity in the mouse SMG duct. The evidence includes a DIDS-sensitive, Na+out-independent, electrogentic Cl−-dependent HCO3− influx and Cl−out-dependent HCO3− efflux. By contrast, a work with the main SMG duct concluded low or no AE activity in the mouse duct (30). This discrepancy may be the result of sufficiently high Cl− and/or HCO3− conductance in the previous study (30) which masked the AE activity.

We had a particular interest in evaluating HCO3− conductance in native CFTR-expressing cells and its possible regulation by CFTR. In a recent work, Ishguro et al. (6) reported the intriguing finding that luminal AE mediated HCO3− transport in resting but not cAMP-stimulated guinea pig pancreatic ducts. In addition, CFTR-dependent HCO3− conductance was reported in pancreatic duct cells (35) and in an airway epithelial cell line expressing CFTR (20). This would imply that stimulation of CFTR should dramatically increase the HCO3− conductance of the luminal membrane. In the present work we were unable to obtain evidence in support of such a conclusion. Our results in the mouse are in agreement with the finding that HCO3− conductance is at least 6-fold lower than that of Cl− (20, 35) and the absence of HCO3− conductance in sweat duct (40). Hence, in the presence of physiological Cl− and HCO3− gradients it is not likely that CFTR mediates or modulates the HCO3− conductance in the luminal membrane of the rat and mouse secretory epithelia. Accordingly, the electrogenic component of HCO3− transport in T84 cells (Fig. 1) and SMG ducts (Figs. 4 and 7) was rather small and not affected by inhibitors of CFTR Cl− channel activity (Fig. 1 and not shown). Furthermore, stable and transient transfection of CFTR in NIH 3T3 and HEK 293 cells, respectively, did not increase HCO3− conductance even when the cells were stimulated with forskolin (compare the effect of high K+ in Fig. 10 of Ref. 28 and Figs. 1c and 7 of this paper).

Low luminal HCO3− conductance in the mouse SMG and pancreatic duct cannot be extended to other species and tissues. This is because, most likely, different mechanisms mediate HCO3− transport in different tissues and species. For example, the electrogenic component of HCO3− transport is much higher in the duodenum than in the colon (3) or the SMG (2). The pancreatic juice of the guinea pig contains a much higher HCO3− concentration than that of the rat (1, 6). However, despite this variability we can safely conclude that in tissues secreting HCO3− in a mechanism similar to that of the mouse SMG or the pancreatic ducts (a) HCO3− conductance is of secondary importance in HCO3− secretion, and (b) regulation of luminal AE activity by CFTR may be the major mechanism by which CFTR regulates HCO3− secretion. In this respect it would be of particular significance to test the effect of CFTR stimulation on luminal AE activity and HCO3− conductance in guinea pig SMG and pancreatic ducts.

The major finding of the present work was extending the finding of regulation of AE activity by CFTR to native CFTR-expressing tissues such as the SMG and pancreas. Even though the increase in AE activity after forskolin stimulation of SMG duct was not as prominent as that observed in the transfected cell lines (28), it could be clearly demonstrated even when the cells were not depolarized (Fig. 5). It is important to note that the extent of stimulation of AE activity by CFTR in SMG ducts may be significantly underestimated. Studies on the rat SMG duct showed the presence of AE activity in both the BLM and LM (18). Expression of CFTR in the LM of SMG ducts (16) indicates that CFTR should stimulate AE activity present in the LM but not in the BLM of SMG ducts. Hence, AE activity in the BLM, although lower than that in the LM (18), increases the background against which the stimulation of AE activity by CFTR is evaluated. This reasoning is reinforced by the finding...
that CFTR stimulates the luminal but not the basolateral AE activity of the pancreatic duct. Notably, in the SMG duct, when the AE activity of both membranes contributed to the measurement, stimulation of CFTR increased AE activity by about 43% (Fig. 7). Under the same depolarized conditions CFTR increased luminal AE activity of the pancreatic duct by about 96% (Fig. 8).

The exclusive expression of CFTR in the luminal membrane of the SMG duct and other CFTR-expressing cells indicates that also in these tissues CFTR regulates the luminal AE. To date the protein responsible for the luminal AE activity of CFTR-expressing cells has not been identified. Good immunocytochemical evidence indicates that the housekeeping AE2 is expressed exclusively in the basolateral membrane of SMG duct and acinar cells (19). This excludes AE2 as the isoform regulated by CFTR. It has been suggested that a variant of AE1 is expressed alternatively in the basolateral or luminal membrane of intercalated cells of collecting duct based on the metabolic state of the animal (41). However, preliminary reverse transcriptase-polymerase chain reaction analysis of the AE isoforms expressed in the cell lines used in the present paper and in Ref. 28 indicates that these cells express only AE2 and AE3 (not shown). Hence, it is possible that CFTR regulates AE3 and that AE3 is the isoform expressed in the luminal membrane of the SMG duct. Another alternative is that a protein other than the known AE isoforms mediates the luminal AE activity. Using expression systems we are attempting to test the effect of CFTR on the activity of each AE isoform.

Our findings provide new insight into the mechanisms of fluid and electrolyte secretion by CFTR-expressing cells and into the pathophysiology of cystic fibrosis. An important function of CFTR-expressing cells is the secretion of HCO₃⁻ (1–3, 7, 9, 12, 25–27). HCO₃⁻ is a chaotropic anion that is commonly used to dissolve, and thus, strip membranes of peripheral proteins. Moreover, HCO₃⁻ regulates the pH of biological fluids. Fluids secreted by CFTR-expressing cells are rich in proteins, in particular mucins (1–3, 7, 25–27). Mucin solubility may be increased in high HCO₃⁻, high pH fluids (42). In the special case of the exocrine pancreas, the high pH is also needed to prevent premature activation of harmful digestive enzymes (1). Impaired HCO₃⁻ secretion may cause precipitation of mucins and thus play a major role in obstruction of almost all ductal systems in cystic fibrosis (43). Every model of luminal HCO₃⁻ secretion in CFTR-expressing cells suggests that AE activity mediates the electroneutral portion of HCO₃⁻ secretion (1–3, 7). By demonstrating regulation of AE activity by an activated CFTR, our findings indicate that CFTR directly regulates the entire process of HCO₃⁻ secretion. Furthermore, the suggested regulation of luminal Na⁺ channel by CFTR (44) allows the regulation of Na⁺ absorption by CFTR. Several CFTR-expressing cells, such as the SMG duct, while absorbing Na⁺ secrete isotonic K⁺ (2). The luminal pathway responsible for K⁺ efflux is believed to be an inward rectifier K⁺ channel such as ROMK II in the kidney (3). CFTR has also been implicated as a regulator of ROMK II (45). Hence, CFTR must be viewed as a global regulator of epithelial fluid and electrolyte transport through its ability to function as a Cl⁻ channel (46) and regulate Cl⁻ absorption, regulate the epithelial Na⁺ channel to regulate Na⁺ absorption, regulate the luminal K⁺ channel to regulate K⁺ secretion, and regulate AE activity to regulate HCO₃⁻ secretion.

The global role of CFTR indicates that its action is not likely to be replaced solely by activation of luminal Cl⁻ channels through stimulation of selective P₂ purinoceptors. Again, the finding that cholinerigic stimulation of intestinal HCO₃⁻ secretion is abolished in the intestines of CFTR −/− mice (24) supports the global role of CFTR in epithelial fluid and electrolyte secretion. Cholinerigic stimulation is expected to activate the luminal Ca²⁺-activated Cl⁻ channel found in many epithelial cells (47). Activation of this Cl⁻ channel was not sufficient to cause normal HCO₃⁻ secretion in cholinergically stimulated intestinal epithelium (24). Hence, although stimulation of luminal purinoceptors may induce Cl⁻ secretion, it is not likely to be sufficient in itself in alleviating the symptoms of cystic fibrosis. Thus, improved expression of CFTR in the luminal membrane seems to be the best option in this endeavor.

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