Functional coupling of apical Cl⁻/HCO₃⁻ exchange by guinea pig interlobular pancreatic duct

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Functional coupling of apical Cl⁻/HCO₃⁻ exchange with CFTR in stimulated HCO₃⁻ secretion by guinea pig interlobular pancreatic duct. Am J Physiol Gastrointest Liver Physiol 296: G1307–G1317, 2009. First published April 2, 2009; doi:10.1152/ajpgi.90697.2008.—Pancreatic ductal epithelium produces a HCO₃⁻-rich fluid. HCO₃⁻ transport across ductal apical membranes has been proposed to be mediated by both SLC26-mediated Cl⁻/HCO₃⁻ exchange and CFTR-mediated HCO₃⁻ conductance, with proportional contributions determined in part by axial changes in gene expression and luminal anion composition. In this study we investigated the characteristics of apical Cl⁻/HCO₃⁻ exchange and its functional interaction with CFTR activity in isolated interlobular ducts of guinea pig pancreas. BCECF-loaded epithelial cells of luminally microperfused ducts were alkalized by acetate prepulse or by luminal Cl⁻ efflux and its functional interaction with CFTR in isolated interlobular ducts of guinea pig pancreas. BCECF-loaded epithelial cells of luminally microperfused ducts were alkalized by acetate prepulse or by luminal Cl⁻ removal or by luminal Cl⁻ removal in the presence of HCO₃⁻-CO₂. Intracellular pH recovery upon luminal Cl⁻ restoration (nominal Cl⁻/HCO₃⁻ exchange) in cAMP-stimulated ducts was largely inhibited by luminal dihydro-DIDS (H-DIDS), accelerated by luminal CFTR inhibitor inh-172 (CFTRinh-172), and was insensitive to elevated bath K⁺ concentration. Luminal introduction of CFTRinh-172 into sealed duct lumens containing BCECF-dextran in HCO₃⁻-free, Cl⁻-rich solution enhanced cAMP-stimulated HCO₃⁻ secretion, as calculated from changes in luminal pH and volume. Luminal Cl⁻ removal produced, after a transient small depolarization, sustained cell hyperpolarization of ~15 mV consistent with electrically parallel CCl⁻/HCO₃⁻ exchange. The hyperpolarization was inhibited by H-DIDS and potentiated by CFTRinh-172. Interlobular ducts expressed mRNAs encoding CFTR, Slc26a6, and Slc26a3, as detected by RT-PCR. Thus Cl⁻-dependent apical HCO₃⁻ secretion in pancreatic duct is mediated predominantly by an Slc26a6-like Cl⁻/HCO₃⁻ exchanger and is accelerated by inhibition of CFTR. This study demonstrates functional coupling between CFTR and Slc26a6-like Cl⁻/HCO₃⁻ exchange activity in apical membrane of guinea pig pancreatic interlobular duct.

bicarbonate; Slc26; cystic fibrosis transmembrane conductance regulator; forskolin; H₂DIDS

The pancreatic ducts of human and guinea pig secrete a HCO₃⁻-rich fluid in response to stimulation by the adenylyl cyclase-coupled hormone secretin. The Cl⁻-rich secretion of pancreatic acinar cells is modified as it flows along the pancreatic ductal system, ultimately producing pancreatic juice with final concentrations of ~140 mM HCO₃⁻ and ~20 mM Cl⁻ (31). In the guinea pig pancreatic duct, the uptake of bicarbonate across the basolateral membrane is mediated by Na⁺-/HCO₃⁻ cotransport (NBC1) and by Na⁺/H⁺ exchange (19). HCO₃⁻ efflux across the apical membrane has been proposed to be mediated by CFTR, in concert with apical Cl⁻/HCO₃⁻ exchanger activity (9).

Proximal pancreatic duct HCO₃⁻ secretion by apical Cl⁻/HCO₃⁻ exchange is favored by the high luminal Cl⁻ concentration resulting from pancreatic acinar Cl⁻ secretion. However, since this proximal fluid flows distally toward the ampulla terminus of the duct, its Cl⁻ concentration progressively decreases in parallel with a gradual increase in its HCO₃⁻ concentration. These inverse changes in Cl⁻ and HCO₃⁻ concentrations are predicted to alter relative contributions of apical Cl⁻/HCO₃⁻ exchange and CFTR-mediated HCO₃⁻ conductance and have been suggested to reflect axial variation in expression and regulation of these transport activities (18). The Cl⁻/HCO₃⁻ exchangers Slc26a3 and Slc26a6 have been detected in mouse pancreatic duct and in the human pancreatic cell line CFPAC-1 (10, 22), leading to the proposal that Cl⁻-dependent HCO₃⁻ secretion by pancreatic proximal ducts represents combined activities of Slc26a3 and Slc26a3 anion exchangers, positively regulated by activated CFTR, with reciprocal positive regulation of CFTR by the anion exchangers (4, 5, 22, 23). However, a study in native mouse pancreatic duct suggests negative regulation of CFTR by Slc26a6 (43).

Secretin-stimulated HCO₃⁻ secretion in guinea pig pancreatic duct is not dependent on elevated luminal [Cl⁻] (21) (brackets denote concentration) and occurs even in the presence of luminal fluid containing 125 mM HCO₃⁻ and 23 mM Cl⁻ (17). As luminal [Cl⁻] falls to or below this level (with corresponding elevation of [HCO₃⁻]), CFTR Cl⁻ permeability may fall while that for HCO₃⁻ may rise (34). Measurements of membrane potential (Vₘ) and intracellular pH (pHᵢ) in luminal perfused interlobular ducts from guinea pig pancreas suggest that CFTR HCO₃⁻ conductance can account for observed levels of stimulated bicarbonate secretion (15, 18, 20). A computational model of pancreatic duct HCO₃⁻ secretion predicted for similar conditions that ~94% of HCO₃⁻ efflux across the apical membrane was mediated by HCO₃⁻ conductance (36), although the model did not consider contributions from electrogenic Cl⁻/HCO₃⁻ exchange. Thus the HCO₃⁻ conductance of CFTR could provide the main route for apical HCO₃⁻ secretion in distal pancreatic ducts from species in which, like human and guinea pig, pancreatic juice contains high concentrations of HCO₃⁻ (~140 mM).

Studies of the role of SLC26-mediated Cl⁻/HCO₃⁻ exchange activity in pancreatic ductal HCO₃⁻ secretion have to date been limited to genetically tractable mouse models. In isolated, perfused mouse interlobular pancreatic duct depleted of intra-
cellular Cl\(^-\), genetic absence of Slc26a6 was associated with 45% reduction in secretin-stimulated luminal Cl\(^-\)/HCO\(_3\)\(^-\) exchange as measured during luminal Cl\(^-\) restoration (13). However, in resealed mouse pancreatic duct segments, genetic absence of Slc26a6 was associated with 70% elevation of basal Cl\(^-\)/HCO\(_3\)\(^-\) exchange activity (43). In addition, it was suggested that CFTR activity could be inhibited by Slc26a6 in the unstimulated mouse pancreatic duct (43). However, the low HCO\(_3\)\(^-\) concentration of mouse pancreatic juice (~40 mM) contrasts to ~140 mM HCO\(_3\)\(^-\) in pancreatic juice of human and guinea pig (1), suggesting species-specific mechanisms of apical HCO\(_3\)\(^-\) transport and/or regulation. Thus identification of apical Cl\(^-\)/HCO\(_3\)\(^-\) exchangers in guinea pig pancreatic duct and assessment of their interactions with CFTR may enhance our understanding of normal human pancreatic HCO\(_3\)\(^-\) secretion and its alterations in disease states.

We have measured pharmacologically defined activities of apical Cl\(^-\)/HCO\(_3\)\(^-\) exchange and their response to CFTR inhibition in microperfused, forskolin-stimulated, interlobular ducts isolated from guinea pig pancreas. Because previous studies of apical Cl\(^-\)/HCO\(_3\)\(^-\) exchange demonstrated that luminal H2DIDS decreased intracellular [Cl\(^-\)] to a greater degree in cAMP-stimulated than in unstimulated ducts (15), we studied only forskolin-stimulated ducts. The effect of CFTR inhibition on Cl\(^-\)-dependent HCO\(_3\)\(^-\) secretion into resealed interlobular duct fragments was also examined. We demonstrate a functional coupling between CFTR and Slc26a6-like apical anion exchange activity in isolated pancreatic interlobular duct under physiological conditions, such that CFTR inhibition increases apical Cl\(^-\)/HCO\(_3\)\(^-\) exchange activity.

**MATERIALS AND METHODS**

**Isolation and culture of interlobular ducts.** Interlobular ducts (diameter 100–150 μm, length 800–1,200 μm) were isolated as described previously (19), in accordance with a protocol approved by the Nagoya University Committee on Ethical Animal Use for Experiments (21). Female Hartley guinea pigs (300–350 g) were killed by cervical dislocation. The pancreas was then removed, sliced into fragments of ~1 mm\(^3\), and digested with collagenase and hyaluronidase at 37°C for 25 min, with an additional 20 min in fresh solution.

Interlobular duct segments were microdissected with sharpened needles under a dissecting microscope, typically yielding 15–20 duct segments per pancreas. These duct segments are believed to represent second degree branches off the main pancreatic duct. The ducts were cultured overnight at 37°C as described previously (19).

**Solutions.** Standard HEPES buffer contained (in mM) 140 NaCl, 5 KCl, 1 CaCl\(_2\), 1 MgCl\(_2\), 10 d-glucose, and 10 HEPES. Standard HCO\(_3\)-buffered solutions contained (in mM) 115 NaCl, 25 NaHCO\(_3\), 5 KCl, 1 CaCl\(_2\), 1 MgCl\(_2\), and 10 d-glucose, equilibrated with 95% O\(_2\)-5% CO\(_2\). In the absence of CO\(_2\)/HCO\(_3\), pH was measured ratiometrically in interlobular ducts by use of the pH-sensitive fluorophore 2′,7′-bis(2-carboxyethyl)-5-(6)-carboxyfluorescein (BCECF-AM; Molecular Probes, Eugene, OR).

**Measurement of intracellular pH.** Intracellular pH (pHi) was measured ratiometrically in interlobular duct cells by use of the pH-sensitive fluorophore 2′,7′-bis(2-carboxyethyl)-5-(6)-carboxyfluorescein, acetoxymethyl ester (BCECF-AM; Molecular Probes, Eugene, OR). Isolated interlobular ducts were incubated in 2 μM BCECF-AM at room temperature for 10 min. Small regions of BCECF-loaded duct epithelium were excited alternately at 440 and 490 nm, and fluorescence emission was measured at 530 nm (F\(_{440}\) and F\(_{490}\)) by use of an inverted microscope (Olympus, Japan) adapted for epifluorescence.

**Calculation of H\(^+\)-equivalent influx.** Net H\(^+\)-equivalent influx (J\(_{H^+}\)) via apical anion exchangers was estimated from the decrease in pHi following either an acetate prepulse or readdition of luminal Cl\(^-\). Net acid influx was calculated using the equation:

\[
J_{H^+} = \beta_{int}(pHi) \times dpHi/dt
\]

where \(\beta_{int}\) is the total buffering capacity [= intrinsic buffering capacity of the guinea pig pancreatic duct cell (\(\beta_{int}\)) + buffering capacity of the CO\(_2\)/HCO\(_3\) system (\(\beta_{int}\))] and is a function of pHi. In Fig. 2, apical anion exchange activity was measured in the presence and absence of CO\(_2\)/HCO\(_3\). In the absence of CO\(_2\)/HCO\(_3\), \(\beta_{int} = \beta_{tot}\). In each individual duct, dpHi/dt during sequential experimental maneuvers in the continued presence of CO\(_2\)/HCO\(_3\) was measured at a uniform pHi. In most cases this pHi value was the midpoint of the pH change (ΔpHi) elicited by the maneuver under study and is referred to as the “midpoint pHi value.”

**Measurement of luminal pH and fluid secretory rate in isolated pancreatic ducts.** The pH of the duct lumen (pHi) was estimated by microfluorimetry as described previously (17, 21). The lumen of sealed ducts was punctured with a double-barreled (theta-glass) micropipette. Luminal fluid content was withdrawn and replaced with HCO\(_3\)-free, HEPES-buffered injection solution containing 20 μM BCECF-dextran (70 kDa).

The rate of fluid secretion into the lumen of resealed ducts was measured as previously described (17). Luminal fluorescence images were acquired at 1-min intervals via a charge-coupled device camera and transformed to binary images by using ARGUS 50 software (Hamamatsu Photonics, Hamamatsu, Japan). To determine secretory rate, initial values for the length (L\(_0\)), diameter (2R\(_0\)), and projected area (A\(_0\)) of the duct lumen were measured in the first image of the series. The initial volume of the duct lumen (V\(_0\)) was calculated, assuming cylindrical geometry, as πR\(_0^2\)L\(_0\). The luminal surface area of the epithelium was taken to be 2πR\(_0\)L\(_0\). Relative volume (V/V\(_0\)) was calculated from relative area (A/A\(_0\)) from the relationship V/V\(_0\) = (A/A\(_0\))^2/2. Fluid secretion rates were calculated at 1-min intervals from increments in duct volume and expressed as secretion rates per unit area of luminal epithelium (nl/min·mm\(^-2\)·mm\(^-2\)). The values of L\(_0\) and R\(_0\) were 459 ± 23 and 69 ± 3 μm, respectively (n = 14, means ± SE). HCO\(_3\) concentration in the lumen ([HCO\(_3\)\(^-\)]) was estimated from pHi, with assumed values for CO\(_2\) solubility of 0.03 mM/mM Hg and pK of the HCO\(_3\)-CO\(_2\)-buffer system of 6.1 (17). The rate of HCO\(_3\) secretion into resealed duct lumens was calculated from the fluid secretory rate and changes in [HCO\(_3\)\(^-\)].
Pancreatic Duct Bicarbonate Secretion

Throughout the experiment, the initial luminal solution of nominally HCO₃⁻ was fused with bath solution containing 124 mM Cl⁻. A 4-min luminal exposure to 80 mM sodium acetate (Cl⁻/HCO₃⁻ exchange, period a–b) increased pHᵢ by 0.074 ± 0.04 pH units (n = 13) over a 4-min period, followed by complete pHᵢ recovery upon readdition of luminal Cl⁻ (Fig. 1A, b–c). Addition of 80 mM sodium acetate to the luminal perfusate led to initial acidification (Fig. 1A, d–e), followed by a large intracellular alkalinization (Fig. 1A, e–f) upon subsequent acetate removal (mean peak value of pHᵢ following acetate removal was pH 7.82 ± 0.07, n = 10). pHᵢ recovery was slower (P < 0.02) in the absence of luminal Cl⁻ (Fig. 1A, f–g; glucuronate replacement; Jₜᵢ = 6.6 ± 1.7 mM/min, n = 7) than in its presence (Fig. 1B, Jₜᵢ = 15.0 ± 2.3 mM/min, n = 9). Subsequent readdition of luminal Cl⁻ (Fig. 1A, g–h) prompted intracellular acidification at rates identical to those observed during initial recovery conditions (Fig. 1A, b–c). These two protocols show pHᵢ recovery from alkaline load independent of the method of base loading. The data are consistent with the presence of apical Cl⁻/HCO₃⁻ or Cl⁻/OH⁻ exchange (or the formally equivalent H⁺/Cl⁻ cotransport).

Figure 2A compares pHᵢ recovery from an acetate-induced alkalinosis in CO₂/HCO₃⁻ buffer compared with that in nominally HCO₃⁻-free, HEPES-buffered solution in ducts stimulated with forskolin. Basolateral H₂DIDS was applied to minimize the contributions to pHᵢ recovery of basolateral Cl⁻/HCO₃⁻ exchange or Na⁺–H⁺–HCO₃⁻ cotransport [the guinea pig duct basolateral membrane lacks Na⁺–dependent Cl⁻/HCO₃⁻ exchange (19)]. The initial pHᵢ recovery (base efflux) following acetate prepulse was considerably faster in CO₂/HCO₃⁻ buffer than in its absence (Fig. 2B; *P < 0.02, Student’s unpaired t-test).

**RESULTS**

Apical Cl⁻/HCO₃⁻ exchange in microperfused interlobular pancreatic ducts. Figure 1 illustrates two experimental protocols for measurement of cAMP-activated apical Cl⁻/HCO₃⁻ exchange. To maximize the HCO₃⁻ and Cl⁻ gradients across the apical membrane, an isolated interlobular duct was superfused with bath solution containing 124 mM Cl⁻ and 25 mM HCO₃⁻–5% CO₂, and the duct lumen was perfused with nominally HCO₃⁻-free, HEPES-buffered solution containing 124 mM Cl⁻. Under these conditions resting pHᵢ was 7.32 ± 0.03 (n = 22). In the presence of 1 μM forskolin, removal of Cl⁻ from the luminal solution by replacement with glucuronate (Fig. 1A, a–b) increased pHᵢ by 0.074 ± 0.04 pH units (n = 13) over a 4-min period, followed by complete pHᵢ recovery upon readdition of luminal Cl⁻ (Fig. 1A, b–c). Addition of 80 mM sodium acetate to the luminal perfusate led to initial acidification (Fig. 1A, d–e), followed by a large intracellular alkalinization (Fig. 1A, e–f) upon subsequent acetate removal (mean peak value of pHᵢ following acetate removal was pH 7.82 ± 0.07, n = 10). pHᵢ recovery was slower (P < 0.02) in the absence of luminal Cl⁻ (Fig. 1A, f–g; glucuronate replacement; Jₜᵢ = 6.6 ± 1.7 mM/min, n = 7) than in its presence (Fig. 1B, Jₜᵢ = 15.0 ± 2.3 mM/min, n = 9). Subsequent readdition of luminal Cl⁻ (Fig. 1A, g–h) prompted intracellular acidification at rates identical to those observed during initial recovery conditions (Fig. 1A, b–c). These two protocols show pHᵢ recovery from alkaline load independent of the method of base loading. The data are consistent with the presence of apical Cl⁻/HCO₃⁻ or Cl⁻/OH⁻ exchange (or the formally equivalent H⁺/Cl⁻ cotransport).

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These data, together with those of Fig. 1, document the presence of apical Cl⁻/HCO₃⁻ exchange activity in isolated guinea pig interlobular ducts, expanding on others’ observations (41).

**Effect of luminal inhibitors on pHᵢ recovery from an intracellular alkaline load in microperfused interlobular pancreatic ducts.** Recent evidence from Slc26a6⁻/⁻ knockout mice suggests Slc26a6 and Slc26a3 polypeptides as candidate apical Cl⁻/HCO₃⁻ exchangers in microperfused mouse pancreatic interlobular ducts (13, 43). These two Slc26 anion exchangers differ in their sensitivities to inhibition by stilbene disulfonates (3, 4, 28). We therefore assessed sensitivity of apical Cl⁻/HCO₃⁻ exchange activity to H₂DIDS. Figure 3A shows that in the presence of 25 mM HCO₃⁻ in bath and lumen, bath Cl⁻ removal from forskolin-stimulated ducts increased pHᵢ 0.18 ± 0.02 pH units (n = 15) in 2–3 min because of reversal of basolateral Cl⁻/HCO₃⁻ exchange. The rate of base loading (Jₚᵢ) following bath Cl⁻ removal was 18.8 ± 3.4 mM/min (n = 12). pH recovery from alkalinization (Jₚᵢ) following readdition of bath Cl⁻ was 19.9 ± 4.7 mM/min (n = 12). In contrast, luminal Cl⁻ removal caused a larger pHᵢ increase of 0.25 ± 0.02 pH units (n = 22) in 2–3 min, due to reversal of apical Cl⁻/HCO₃⁻ exchange activity. The rate of base loading (Jₚᵢ) following luminal Cl⁻ removal was 24.2 ± 2.4 mM/min (n = 22). Subsequent readDITION of luminal Cl⁻ prompted influx of acid equivalents (Jₚᵢ) at 21.5 ± 2.8 mM/min (n = 22; P > 0.05, Student’s paired t-test for base loading rate vs. acid influx rate measured at the same pHᵢ). As shown in Fig. 3A and summarized in Fig. 3B, luminal addition of H₂DIDS (200 μM) coincident with luminal Cl⁻ restoration slowed pHᵢ recovery from a base load by ~80% (n = 5, P < 0.02). These data showing H₂DIDS-sensitive, apical Cl⁻/HCO₃⁻ exchange are consistent with the pharmacological properties of mouse and human Slc26a6.

In the presence of high luminal [Cl⁻], apical Cl⁻/HCO₃⁻ exchange has been proposed to mediate most HCO₃⁻ secretion, with CFTR HCO₃⁻ conductance playing a minor role (1, 37). In this setting CFTR is thought to support luminal Cl⁻-dependent
HCO3 secretion by recycling intracellular Cl across the apical membrane (30, 45). Therefore, we examined the effect of CFTR inhibition on pHi recovery following a base load, using luminal application of CFTRinh-172, a specific CFTR inhibitor (27) that does not inhibit Cl /HCO3 exchange in CFTR-transduced CFPAC-1 cells (33). Figure 4A shows a pHi trace from a representative duct luminally perfused with 25 mM HCO3 solution in a 25 mM HCO3 bath containing 1 µM forskolin. Removal of luminal Cl- rapidly alkalized the duct. Subsequent luminal Cl- restoration induced pHi recovery from this base load significantly more rapidly (P < 0.02, paired experiments) in the presence of CFTRinh-172 (JH = 23.4 ± 5.3, n = 12) than in the drug’s subsequent absence (JH = 13.27 ± 2.2, n = 12; Fig. 4B). Thus inhibition of CFTR accelerated luminal Cl- dependent HCO3 efflux across the apical membrane (Cl /HCO3 exchange) by ~80% in alkaline-loaded stimulated ducts.

We next examined the combined effects of luminal H2DIDS and CFTRinh-172 on luminal Cl- dependent pHi recovery from alkaline load in the presence of 25 mM HCO3 in both bath and lumen. The pHi trace from a representative duct in Fig. 5A and the summarized paired duct data in Fig. 5B show that luminal H2DIDS inhibited the CFTRinh-172-accelerated rate of pHi recovery from alkaline load (JH = 3.7 ± 0.7 mM/min, n = 7; P < 0.02, Student’s paired t-test) compared with that in the absence of H2DIDS and inh-172 (JH = 17.4 ± 2.3 mM/min, n = 7). As summarized in Fig. 5C, which shows normalized pHi recovery data following luminal Cl- restoration taken from Figs. 3A, 4A, and 5A, CFTRinh-172 enhanced H2DIDS-sensitive apical Cl- /HCO3 exchange. These data suggest that CFTR and Slc26a6-like, H2DIDS-sensitive Cl- /HCO3 exchange are functionally coupled in the duct apical membrane.

Effects of luminal CFTRinh-172 on Cl- dependent HCO3 secretion in cAMP-stimulated interlobular pancreatic ducts.

To investigate functional coupling between CFTR and Cl- /HCO3 exchange under more physiological conditions, we examined the effects of luminally injected CFTRinh-172 on Cl- dependent HCO3 secretion in sealed ducts. Changes in pHi and volume were simultaneously monitored and rates of fluid and HCO3 secretion were calculated. The lumens of sealed ducts were micropunctured and filled with HCO3-free, HEPEES-buffered 149 mM Cl- containing BCECF-dextran (20 µM). The sealed ducts were initially superfused with the same HCO3- free, HEPEES-buffered solution and maximally stimulated by 1 µM bath forskolin. The bath solution was then switched to HCO3 -CO2-buffered solution in the continued presence of forskolin. pHi transiently decreased due to CO2 diffusion into the lumen and then subsequently increased due to HCO3 secretion (17), reaching pHi values of 7.72 ± 0.02 in the absence and 7.86 ± 0.04 in the presence of 10 µM luminal CFTRinh-172 after 10 min (Fig. 6A; n = 7, P < 0.01).

Fluid secretion rapidly increased to stable values (Fig. 6B), with rates of 1.7 ± 0.1 nl·min⁻¹·mm⁻² in the absence and 1.3 ± 0.1 nl·min⁻¹·mm⁻² in the presence of CFTRinh-172 (Fig. 6C; n = 7, P < 0.05). Calculated HCO3 secretion rates during the 10-min perfusion with HCO3 -CO2 buffer were 2.65 ± 0.15 nEq/mm in the absence and 3.53 ± 0.23 nEq/mm in the presence of CFTRinh-172 (Fig. 6D, n = 7, P < 0.01). Thus luminal CFTRinh-172 significantly enhanced HCO3 secretion while inhibiting net fluid secretion in the presence of high luminal [Cl-]. The decreased fluid secretion likely reflected inhibition of CFTR-mediated Cl- secretion. Previous studies suggest that HCO3 secretion is mediated largely by H2DIDS-sensitive Cl- /HCO3 exchange in the presence of high luminal [Cl-] (17, 21). The present data suggest that inhibition of CFTR activity enhances H2DIDS-sensitive apical Cl- /HCO3 exchange under physiological conditions in which Cl- dependent HCO3 secretion is thought to occur in vivo.

Effect of Vm on apical Cl- /HCO3 exchange in microperfused interlobular pancreatic ducts. Strong evidence favors an electrogenic mechanism of CI- /2HCO3 exchange for recombinant mouse Slc26a6 (35), but DIDS-sensitive electroneutral monovalent anion exchange by recombinant mouse Slc26a6 and human SLC26A6 has also been observed (3, 6). Consistent with the latter mechanism, DIDS-sensitive SLC26A6-like Cl- /HCO3 exchange in CFTR-transduced human CFPAC-1 pancreatic duct epithelial cells (10, 33) and in mouse NIH 3T3 fibroblasts and human HEK-293 cells (26) was insensitive to bath [K+] -induced changes in Vm. In addition, net secretion of HCO3 was equivalent to net reabsorption of Cl- in resealed

Fig. 4. Luminal exposure to a CFTR inhibitor accelerates pHi recovery from a base load in microperfused, forskolin-stimulated ducts. A: bath and lumen were separately perfused with the standard CO2/HCO3 solution, with 1 µM bath forskolin present throughout the experiment. Duct cell pHi, was measured during sequential periods of luminal Cl- removal and restoration with the bath continuously superfused with Cl- free solutions, in the absence and subsequent presence of 10 µM CFTR inhibitor inh-172 (CFTRinh-172). B: 12 separate ducts similar to that in A reveal higher mean JH CO2 acid equivalent influx upon luminal Cl- restoration in the presence of inh-172 (open bar) than in its absence (solid bar). For ducts exposed to 4 sequential cycles of Cl- removal and restoration as A, data from the first 2 cycles were included in the pooled data presented in B. Values are means ± SE from paired experiments, *P < 0.05, Student’s paired t-test.

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mouse pancreatic duct segments stimulated with forskolin, and this HCO₃⁻ secretion was completely absent in duct segments from the Slc26a6⁻/­⁻ mouse (45).

To test Vₘ dependence of apical membrane Cl⁻/HCO₃⁻ exchange, ducts were subjected to luminal Cl⁻ removal and restoration in the presence of luminal CFTRinh-172, in Cl⁻-free bath solutions of normal and high [K⁺] containing 40 mM Na⁺ along with 500 μM MH2DIDS to minimize basolateral membrane HCO₃⁻ transport. As shown by the pHi trace of Fig. 7A, and summarized in Fig. 7B (paired experiments), apical Cl⁻/HCO₃⁻ exchange rate (acid influx) in the presence of luminal CFTR inhibitor was on average not altered by bath [K⁺] changes between 5 and 100 mM. The rates of pHᵢ recovery from an alkaline load in the presence of CFTRinh-172 in normal K⁺ bath (Fig. 7B, “control” conditions) were indistinguishable from those reported in Fig. 4B, reinforcing the conclusion that inhibition of CFTR activity leads to increased apical Cl⁻/HCO₃⁻ exchange.

Fig. 5. Luminal H₂DIDS completely inhibits the component of apical Cl⁻/HCO₃⁻ exchange activity accelerated by luminal CFTRinh-172. A: CO₂/HCO₃⁻ bath containing 1 μM forskolin was Cl⁻-free throughout the experiment. pHᵢ was recorded during sequential Cl⁻ removal from and restoration to the CO₂/HCO₃⁻-buffered luminal perfusate in the absence and subsequent combined presence of 10 μM CFTRinh-172 and 200 μM H₂DIDS. B: control mean pHᵢ recovery rate (solid bar) compared with CFTRinh-172 plus H₂DIDS (open bar, mean midpoint pHᵢ 7.68 in both absence and presence of drugs); *n = 7, P < 0.02, Student’s paired t-test. C: normalized rate of pHᵢ recovery (expressed as JₚHᵢ) following luminal Cl⁻ restoration in the absence and presence of the indicated luminal inhibitors. Data are taken from Figs. 3B, 4B, and 5B. Rates of pHᵢ recovery from alkaline load (JₚHᵢ) in the presence of inhibitor were normalized for each duct to the paired, previously measured control rate of pHᵢ recovery for that same duct (100% = 15.6 ± 1.5 mM/min). Values are means ± SE from paired experiments; *P < 0.02, Student’s paired t-test.

Fig. 6. Effects of luminal CFTRinh-172 on Cl⁻-dependent HCO₃⁻ secretion in cAMP-stimulated pancreatic duct. Duct lumens were filled with a Cl⁻-rich (149 mM), HCO₃⁻-free, HEPES-buffered solution containing BCECF dextran. The bath was first superfused with HCO₃⁻-free, HEPES-buffered solution containing 1 μM forskolin. After a 5-min period, CO₂/HCO₃⁻ was introduced to the bath solution in the continued presence of forskolin. Luminal pH (pHᵢ) and fluid secretory rate were monitored for 10 min. A: changes in pHᵢ in the absence (solid line) or presence (dashed line) of luminally injected CFTRinh-172 (10 μM): means ± SE of 7 experiments. Thin solid line and thin dashed line indicate SE in 1 direction only, for clarity. B: changes in fluid secretory rate in the absence (open bars) or presence (shaded bars) of luminally injected CFTRinh-172 (10 μM). Means ± SE of 7 experiments. C: fluid secretion calculated in the absence (open bar) and presence (shaded bar) of CFTRinh-172 during 10-min superfusion with HCO₃⁻-CO₂ buffered solution (*P < 0.05, Student’s unpaired t-test). D: HCO₃⁻ secretion across the apical membrane during 10-min superfusion with HCO₃⁻-CO₂-buffered solution in the absence (open bar) and presence (shaded bar) of CFTRinh-172 (*P < 0.01, Student’s unpaired t-test). Means ± SE for 7 experiments in C and D.
Fig. 7. Depolarization by bath [K⁺] elevation does not alter pH, recovery from base load in microperfused ducts. A: CO₂/HCO₃⁻ bath containing 1 μM forskolin and 500 μM H₂DIDS was Cl⁻-free and the lumen contained 10 μM CFTRinh-172 throughout the experiment. pH was recorded during sequential Cl⁻ removal from and restoration to the CO₂/HCO₃⁻-buffered luminal perfusate with 5 or 100 mM bath K⁺. B: luminal Cl⁻-restoration-induced mean pH recovery rate (section a, mean midpoint pH, 7.55) was not inhibited by the depolarizing maneuver of bath K⁺ (section b, mean midpoint pH, 7.60); means ± SE from paired experiments, *P > 0.05, Student’s paired t-test.

Figure 8 tracks duct Vₘ during luminal Cl⁻ removal and restoration in the presence and absence of luminal H₂DIDS (200 μM) or CFTRinh-172 (10 μM). Bath and luminal solutions both contained 25 mM HCO₃⁻-5% CO₂. The basolateral membrane was impaled by microelectrodes and intracellular potential was measured in reference to the bath. We assume that basolateral Vₑ was likely close in magnitude to apical Vₑ, since the transepithelial potential of the stimulated, perfused rat pancreatic duct was < 5 mV (30). Luminal exposure to either H₂DIDS (Fig. 8A) or CFTRinh-172 (Fig. 8B) resulted in a small hyperpolarization (~4.1 ± 0.9 mV, n = 5; ~4.0 ± 0.3 mV, n = 5, respectively). As shown in Fig. 8, A, B, and C, removal of luminal Cl⁻ resulted in a transient depolarization, followed by a hyperpolarization of the duct by ~15.0 ± 1.0 mV (n = 12). Restoration of Cl⁻ to the luminal perfusate reversed these changes. The transient depolarization was partially inhibited by luminal CFTRinh-172 (Fig. 8B) and thus probably reflected Cl⁻ efflux via CFTR. The subsequent hyperpolarization might be explained by depletion of intracellular Cl⁻ (with Vₑ shift toward the K⁺ equilibrium potential) and/or by 1Cl⁻/2HCO₃⁻ exchange across the apical membrane. Figure 8A shows that the hyperpolarization was attenuated ~44% by luminal H₂DIDS (to -8.4 ± 0.8 mV, n = 5; P < 0.01, Fig. 8D), suggesting that H₂DIDS-sensitive apical Cl⁻/HCO₃⁻ exchange contributes to the hyperpolarization. In contrast, CFTR inhibition by luminal application of CFTRinh-172 enhanced the hyperpolarization by ~45% to ~21.8 ± 2.5 mV (n = 5;
P < 0.05 Figs. 8, B and D) and this enhancement was also inhibited by luminal H2-DIDS (Fig. 8, C and D). These data reveal an H2-DIDS-sensitive hyperpolarizing activity in response to luminal Cl− removal that is consistent with electrogenic Cl−/HCO3− exchange across the apical membrane. The data also confirm that inhibition of CFTR accelerates apical Cl−/HCO3− exchange.

Expression of slc26a3, slc26a6, and CFTR mRNAs in guinea pig interlobular pancreatic duct. Figure 9 shows sequence-confirmed cDNAs encoding Slc26a3, Slc26a6, Cfrtr, and Gapdh, as amplified by RT-PCR from total RNAs of isolated interlobular pancreatic duct, kidney, and colon of guinea pig. The data confirm that interlobular ducts from guinea pig pancreas express mRNAs encoding not only Cfrtr, but also the apical membrane anion exchangers Slc26a3 and Slc26a6, as has been observed in mouse pancreas (13).

DISCUSSION

We have identified in guinea pig interlobular pancreatic duct a H2-DIDS-sensitive, Cl−/HCO3− exchange activity that mediates the major component of forskolin-stimulated, Cl−-dependent HCO3− efflux across the apical membrane and a smaller component of apical Cl−/HCO3− exchange activity insensitive to 200 μM H2-DIDS. The H2-DIDS-sensitive fraction of Cl−/ HCO3− exchange was accelerated by inhibition of CFTR. In addition, we have shown that luminal inhibition of CFTR decreased fluid secretion but increased net apical HCO3− secretion into Cl−-rich luminal fluid. Guinea pig pancreatic duct expressed mRNAs encoding Slc26a3, Slc26a6, and Cfrtr. Luminal Cl− removal reversibly hyperpolarized duct cells in a manner partially attenuated by H2-DIDS, consistent with electrogenic apical membrane 1Cl−/2HCO3− exchange. However, pHi recovery from an alkaline load upon restoration of luminal Cl− (apical Cl−/HCO3− exchange activity) was on average insensitive to depolarization by bath exposure to 100 mM K+.

The data together suggest that CFTR and H2-DIDS-sensitive Cl−/HCO3− exchange are functionally coupled in the apical membrane of pancreatic duct cells to mediate fluid and HCO3− secretion. On the basis of pharmacological properties of the recombinant mouse and human orthologs, the component of apical Cl−/HCO3− exchange sensitive to 200 μM H2-DIDS may be mediated by Slc26a6, whereas the insensitive component may be mediated by Slc26a3.

Identification of apical Cl−/HCO3− exchange activity in interlobular pancreatic duct of guinea pig. Although HCO3− and Cl− transport by the microperfused pancreatic duct of guinea pig has been studied (15–17), direct study of regulated luminal Cl−/HCO3− exchange by pHi measurement has until recently not been reported. We have detected apical membrane Cl−/base exchange activity in forskolin-stimulated interlobular pancreatic duct. Cl−/HCO3− exchange was severalfold more rapid than Cl−/OH− exchange (Fig. 2) and largely inhibited by luminal 200 μM H2-DIDS. H2-DIDS-sensitive apical Cl−/ HCO3− exchange in guinea pig pancreatic duct was recently shown to be stimulated by bile acids by a mechanism dependent on intracellular Ca2+ increase (41).

mRNAs encoding both apical exchangers Slc26a3 and Slc26a6 have been detected in the mouse pancreatic duct and in the human pancreatic cell line CFPAC-1 (10, 22, 32). Slc26a3 and Slc26a6 mRNAs are also expressed in intact guinea pig interlobular pancreatic ducts (Fig. 8). Among the functional differences noted to date between recombinant Slc26a3 and Slc26a6 are the contrasting DIDS insensitivity of Slc26a3 (4, 28) and the DIDS sensitivity of Slc26a6 (3, 10). Forskolin-stimulated, luminal Cl−-dependent HCO3− efflux following base load was inhibited ~80% by 200 μM H2-DIDS in guinea pig pancreatic ducts (Fig. 3). H2-DIDS similarly inhibited duct cell HCO3− efflux under basal conditions and in response to stimulation by secretin (12). H2-DIDS inhibited both basal and secretin-stimulated fluid secretion into Cl−-rich luminal fluid (17). The apical Cl−/HCO3− exchange activity sensitive to 200 μM H2-DIDS thus resembles Slc26a3 (3, 10), and anion exchange activity insensitive to 200 μM H2-DIDS recalls Slc26a3 (4, 28), with the former contributing the greater portion of Cl−-dependent HCO3− secretion across the apical ductal membrane.

Vm sensitivity of Slc26-like Cl−/HCO3− exchange in guinea pig pancreatic duct. Electrogenic 1Cl−/2HCO3− exchange by Slc26a6 was first suggested by membrane hyperpolarization upon removal of bath Cl− in Xenopus oocytes expressing mouse Slc26a6 (22, 44). Electrogenic anion exchange with a stoichiometry of 1Cl−/2HCO3− was later confirmed by simultaneous measurement of pHi, intracellular Cl− ([Cl−]i), and Vm (35). However, other studies reported Vm-insensitive Cl−/ HCO3− exchange mediated by mouse Slc26a6 and human SLC26A3 (3). Rates of endogenous apical H2-DIDS-sensitive Cl−/HCO3− exchange in CFTR-complemented CFPAC-1 cells (33) and rates of CFTR-activated Cl−/HCO3− exchange in 3T3 and HEK-293 cells (26) were similarly insensitive to depolarization by elevated bath [K+]o. Mouse Slc26a3 was shown to mediate 2Cl−/1HCO3− exchange (35), but Slc26a3-mediated anion exchange with properties of Vm sensitivity or electrogenic activity was not observed by other laboratories (4, 24, 28). Moreover, Slc26a3-mediated Cl−/HCO3− exchange across the apical enterocyte membrane of intact mouse ileum does not exhibit properties of electrogenicity (42).

The Vm hyperpolarization induced by removal of Cl− from HCO3−-containing luminal perfusate (Fig. 8) may reflect depletion of intracellular Cl− (Vm thus approaching toward K+ equilibrium potential). Alternatively, it may reflect 1Cl−/ 2HCO3− exchange across the apical membrane, or a combination of the two. Partial inhibition of hyperpolarization by luminal H2-DIDS suggests that H2-DIDS-sensitive apical 1Cl−/ 2HCO3− exchange by Slc26a6 or a similar activity contributes
substantially to the hyperpolarization. This conclusion was supported further by inhibition of net fluid secretion by CFTRinh-172 of only 24%, demonstrating that net fluid efflux still accompanied apical Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchange in this condition (Fig. 6). The effective HCO\textsubscript{3}\textsuperscript{−} concentration of the fluid secreted in the presence of luminal CFTRinh-172 was estimated from the ratio of measured HCO\textsubscript{3}\textsuperscript{−} secretion and measured fluid secretion to be 283 ± 36 mM. Assuming isotonic salt-water coupling during pancreatic fluid secretion, this value of effective HCO\textsubscript{3}\textsuperscript{−} concentration suggests that ~50% of HCO\textsubscript{3}\textsuperscript{−} secretion is unaccompanied by net fluid flow and is formally mediated by 1:1 exchange with luminal Cl\textsuperscript{−}. Thus an effective HCO\textsubscript{3}\textsuperscript{−} concentration of ~300 mM is compatible with HCO\textsubscript{3}\textsuperscript{−} secretion mediated by 1Cl\textsuperscript{−}/2HCO\textsubscript{3}\textsuperscript{−} exchange.

In contrast, apical Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchange rate (as measured during luminal Cl\textsuperscript{−} removal and restoration) was not significantly affected by exposure to high (100 mM) [K\textsuperscript{+}]\textsuperscript{in} in the bath (Fig. 7), a maneuver that depolarizes V\textsubscript{m} by ~20–30 mV in pancreatic duct of guinea pig (H. Ishiguro, unpublished data) and rat (29). pH\textsubscript{r} recovery from acetate-induced base load in perfused guinea pig interlobular duct was similarly insensitive to high bath K\textsuperscript{+} (n = 10, data not shown). Changes in pH\textsubscript{r} upon removal and restoration of luminal Cl\textsuperscript{−} demonstrated equivalent rates of acid-equivalent transport in the absorptive and secretory directions for apical Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchanger, further consistent with an electroneutral anion exchange process (data not shown).

However, a heterogeneous distribution among isolated duct fragments of electrogenic Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchangers of 1:2 and 2:1 stoichiometries could also produce an averaged insensitivity to high K\textsuperscript{+} depolarization. Alternatively or additionally, the 20–30 mV depolarization elicited by elevated bath [K\textsuperscript{+}] may be too small to influence measurably the observed Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchange activity, in contrast to the 70–140 mV excursions applied to voltage-clamped Xenopus oocytes expressing mouse Slc26a3 and Slc26a6 (7, 35). Considered in this way, the 15 mV changes in duct potential difference elicited by luminal Cl\textsuperscript{−} removal and restoration in the present studies (Fig. 8) might not be expected detectably to modify electrogenic anion exchange rates. Thus the electrogenicity of the apical H\textsubscript{2}DIDS sensitive Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} activity of guinea pig interlobular pancreatic duct remains uncertain.

Interaction of apical Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchange and Cfr in the apical membrane of guinea pig pancreatic duct. Slc26a6-like Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchange may play a major role in HCO\textsubscript{3}\textsuperscript{−} secretion in proximal parts of the pancreatic ductal system characterized by Cl\textsuperscript{−}-rich luminal fluid derived from acinar secretion (18). Activation of apical Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchange is thought to depend on the presence of functional CFTR in pancreatic duct cells. Lee et al. (1999) examined the activities of apical Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchange in luminally perfused main pancreatic duct from wild-type and Cfr\textsubscript{Δ508/Δ508} mice (25). Forskolin stimulation increased the rate of DIDS-sensitive Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchange across the apical membrane in ducts from wild-type mice, but not in ducts from Cfr\textsubscript{Δ508/Δ508} mice. In CFPCR-1 human pancreatic duct epithelial cells lacking CFTR, heterologous CFTR complementation conferred forskolin-stimulated, H\textsubscript{2}DIDS-sensitive (SLC26A6-like) Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchange (33) in the apical membrane (32). Coexpression of recombinant human wild-type but not mutant CFTR conferred or enhanced forskolin-sensitive Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchange activity associated with expression of mouse Slc26a3 and Slc26a6 in HEK-293 cells (22) and with human SLC26A6 (3) and SLC26A3 (4) in Xenopus oocytes. These data suggest that activation of Slc26-mediated Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchange in the apical membrane requires functional and activated CFTR and have been interpreted to reflect direct interaction between the STAS domains of Slc26 anion transporters and the phosphorylated R domain of CFTR (23).

CFTRinh-172 alters CFTR gating by voltage-independent binding to a cytoplasmic nucleotide binding domain (39) or, according to more recent evidence, by direct pore interaction (2). CFTRinh-172 inhibition of CFTR may alter direct or indirect interactions between CFTR and Slc26-like anion exchangers. However, the functional coupling observed in this study does not require such direct molecular interaction. CFTR and Slc26a6 experience similar concentration gradients for Cl\textsuperscript{−} and HCO\textsubscript{3}\textsuperscript{−} at the duct apical membrane and may act synergistically or antagonistically in HCO\textsubscript{3}\textsuperscript{−} secretion. For example, Slc26a6-mediated HCO\textsubscript{3}\textsuperscript{−} efflux upon luminal Cl\textsuperscript{−} restoration may be reduced secondary to rapid Cl\textsuperscript{−} gradient dissipation by Cl\textsuperscript{−} influx through CFTR. In addition, CFTR and (electrogenic) Slc26a6 will experience the same inside-negative V\textsubscript{m} driving force. Thus, in the presence of high luminal [Cl\textsuperscript{−}], Slc26a6-mediated Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchange may be a more energy-efficient form of HCO\textsubscript{3}\textsuperscript{−} secretion than the HCO\textsubscript{3}\textsuperscript{−} conductance of CFTR (8).

In the present study, we used Cl\textsuperscript{−}-rich luminal solutions in microperfused and in sealed ducts to mimic luminal conditions of the proximal duct. (The technical requirements of duct isolation and perfusion required use of interlobular ducts from more distal locations along the duct axis.) We demonstrated with three types of experiments that inhibition of CFTR accelerated Slc26a6-like Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchange across the duct apical membrane. First, luminal application of CFTRinh-172 increased forskolin-stimulated Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchange by ~80% as measured by rate of pH\textsubscript{r} change during luminal Cl\textsuperscript{−} removal and restoration (Fig. 4). This CFTRinh-172-enhanced component of Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchange was completely inhibited by 200 μM luminal H\textsubscript{2}DIDS (Fig. 5), consistent with Slc26a6-mediated Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchange. Second, luminal injected CFTRinh-172 (10 μM) increased forskolin-stimulated HCO\textsubscript{3}\textsuperscript{−} secretion into Cl\textsuperscript{−}-rich luminal fluid (Fig. 6). Third, luminally applied CFTRinh-172 (10 μM) potentiated luminal Cl\textsuperscript{−} removal-induced, 200 μM H\textsubscript{2}DIDS-sensitive hyperpolarization in the presence of HCO\textsubscript{3}\textsuperscript{−} (Fig. 8).

Inhibition of forskolin-stimulated CFTR by inh-172 reduces the rapid but transient depolarization immediately following luminal Cl\textsuperscript{−} removal (Fig. 8), an effect predicted to antagonize activity of an electrogenic apical 1Cl\textsuperscript{−}/2HCO\textsubscript{3}\textsuperscript{−} exchanger. However, inhibition of forskolin-stimulated CFTR by inh-172 is also predicted to elevate duct cell [Cl\textsuperscript{−}], or to reduce its rate of decline during the period of forskolin-stimulated secretion, effects predicted to stimulate apical 1Cl\textsuperscript{−}/2HCO\textsubscript{3}\textsuperscript{−} exchange as measured during luminal Cl\textsuperscript{−} removal. Our data (Figs. 4–6) demonstrate predominance of the latter effect, showing stimulation of ductal apical Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchange by CFTRinh-172. Thus inh-172-induced hyperpolarization in the presence of high luminal [Cl\textsuperscript{−}] may increase HCO\textsubscript{3}\textsuperscript{−} secretion (Fig. 6) by accelerating an apical electrogenic 1Cl\textsuperscript{−}/2HCO\textsubscript{3}\textsuperscript{−} exchange activity, despite the predicted coincident increase in [Cl\textsuperscript{−}]. The lack of Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchange inhibition by bath [K\textsuperscript{+}]
elevation (Fig. 7) and its attendant depolarization of 20–30 mV, in contrast to prediction for a 1Cl−/2HCO3− stoichiometry, remains unexplained in this setting.

Our findings contrast with recent studies in mouse pancreatic duct where inhibition of CFTR with short interfering RNA led to a decrease in secretin-stimulated Slc26a6-mediated Cl−/HCO3− exchange and a decrease in HCO3− efflux into the sealed duct lumen (43). These differences suggest distinct consequences of pharmacological inhibition of CFTR and of decreased CFTR polypeptide abundance. In addition, species differences in regulation and functional activity of apical anion exchange and CFTR channel activity may contribute to the divergent HCO3− concentrations of the stimulated pancreatic ductal secretions produced by mouse and guinea pig.

In the classical model of pancreatic duct HCO3− secretion, apical HCO3− secretion is mediated by Cl−/HCO3− exchange, and CFTR supports HCO3− secretion by recycling to the lumen the Cl− entering the cell in exchange for HCO3− (30). In the present study we have demonstrated two coincident responses to pharmacological inhibition of CFTR: acceleration of apical, Slc26a6-like, Cl−-dependent HCO3− secretion in parallel with decreased volume secretion. The data suggest that, in Cl−-rich acinar fluid conditions of the proximal duct, an Slc26a6-like anion exchanger may be the principal route for HCO3− secretion and can compensate for a low HCO3− permselectivity of CFTR.

Conclusion. Pharmacological inhibition of Cfr accelerates luminal Cl−-dependent HCO3− secretion via Cl−/HCO3− exchange in forskolin-stimulated interlobular duct from guinea pig. The finding that the majority of Cfr-enhanced apical Cl−/HCO3− exchange is H2DIDS sensitive, combined with RT-PCR data from isolated duct, is consistent with an Slc26a6-like anion exchanger functionally coupled to Cfr activity. Our data suggest that this apical Cl−/HCO3− exchanger can compensate for imposed reduction of Cfr activity in stimulated pancreatic interlobular duct. The relative contributions of Cfr, Slc26 Cl−/HCO3− exchangers, and other HCO3− conductances to stimulated pancreatic ductal HCO3− secretion remain to be determined in guinea pig, human, and other species in which pancreatic juice bicarbonate concentration reaches 140 mM. Continued study of pancreatic secretion in these organisms will be important to complement ongoing studies in the mouse, in which the advantage of genetic tractability is accompanied by the considerable disadvantage of reduced HCO3− secretory capacity.

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