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A novel small molecule CFTR inhibitor attenuates HCO_3^- secretion and duodenal ulcer formation in rats

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Akiba, Yasutada, Michael Jung, Samedy Ouk, and Jonathan D. Kaunitz. A novel small molecule CFTR inhibitor attenuates HCO_3^- secretion and duodenal ulcer formation in rats. *Am J Physiol Gastrointest Liver Physiol* 289: G753–G759, 2005. First published May 19, 2005; doi:10.1152/ajpgi.00130.2005.—The cystic fibrosis (CF) transmembrane conductance regulator (CFTR) plays a crucial role in mediating duodenal bicarbonate (HCO_3^-) secretion (DBS). Although impaired DBS is observed in CF mutant mice and in CF patients, which would predict increased ulcer susceptibility, duodenal injury is rarely observed in CF patients and is reduced in CF mutant mice. To explain this apparent paradox, we hypothesized that CFTR dysfunction increases cellular [HCO_3^-] and buffering power. To further test this hypothesis, we examined the effect of a novel, potent, and highly selective CFTR inhibitor, $CFTR_{inh}$-172, on DBS and duodenal ulceration in rats. DBS was measured in situ using a standard loop perfusion model with a pH stat under isoflurane anesthesia. Duodenal ulcers were induced in rats by cysteamine with or without $CFTR_{inh}$-172 pretreatment 1 h before cysteamine. Superfusion of $CFTR_{inh}$-172 (0.1–10 μM) over the duodenal mucosa had no effect on basal DBS but at 10 μM inhibited acid-induced DBS, suggesting that its effect was limited to CFTR activation. Acid-induced DBS was abolished at 1 and 3 h and was reduced 24 h after treatment with $CFTR_{inh}$-172, although basal DBS was increased at 24 h. $CFTR_{inh}$-172 treatment had no effect on gastric acid or HCO_3^- secretion. Duodenal ulcers were observed 24 h after cysteamine treatment but were reduced in $CFTR_{inh}$-172-pretreated rats. $CFTR_{inh}$-172 acutely produces CFTR dysfunction in rodents for up to 24 h. CFTR inhibition reduces acid-induced DBS but also prevents duodenal ulcer formation, supporting our hypothesis that intracellular HCO_3^- may be an important protective mechanism for duodenal epithelial cells.

$CFTR_{inh}$-172; duodenal bicarbonate secretion; duodenal ulcer; cysteamine; luminal acid

THE PROXIMAL DUODENAL MUCOSA is unique in that it is the only leaky gastrointestinal epithelium exposed to gastric acid. Because "leaky" intercellular junctions create an incomplete barrier to acid diffusion, nonstructural defense mechanisms such as mucus secretion, bicarbonate (HCO_3^-) secretion, and blood flow are of prime importance in the defense from luminal acid (13, 23). These defense mechanisms protect the mucosal cells from the intense acid stress continuously present in the gastroduodenal lumen (36, 42). In the presence of luminal acid, the mucosal epithelial cells will be irreversibly acidified, leading to cellular necrosis (28, 37). The major duodenal defense mechanism is epithelial HCO_3^- secretion, which is thought to neutralize luminal acid before it reaches the mucosal cells (5, 12). Although the mechanism of duodenal HCO_3^- secretion (DBS) is not fully understood, it clearly involves the cystic fibrosis (CF) transmembrane conductance regulator (CFTR), because CFTR absence or dysfunction is associated with low levels of basal and stimulated HCO_3^- secretion (8, 39).

CF is the most common monogenetically transmitted lethal disease among populations of northern European ancestry, causing considerable morbidity and early mortality (41). Although luminal acid-related DBS is impaired in CF knockout mice (19) and in CF patients (39), which would predict increased ulcer susceptibility, duodenal ulceration is rarely observed in CF patients (6, 31). To explain this apparent paradox, we hypothesized that the diminished HCO_3^- exit from the epithelial cells associated with CFTR dysfunction increases cellular [HCO_3^-] and hence enhances buffering of cellular H^+. Because HCO_3^- uptake or accumulation into the epithelial cells occurs before DBS (3, 11), inhibition of CFTR should augment cellular [HCO_3^-] and, hence, buffering power. This concept is supported by our observation that the Cl^- channel inhibitor 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB) alkalinizes epithelial cells and reduces cellular acidification and injury due to luminal acid, even though NPPB inhibits acid-induced DBS (4). Baseline intracellular pH is higher in duodenocytes obtained from CF patients (39), also supporting the concept of HCO_3^- "trapping" in cells with defective CFTR function. Furthermore, the duodenum of CF ΔF508 mutant mice is more resistant to cellular acidification and injury in response to luminal acid (17). Nevertheless, we have not yet studied the effect of acute CFTR dysfunction on duodenal ulceration, which would have the advantage of inducing CFTR dysfunction rapidly in adults, minimizing the contribution of compensatory mechanisms. Furthermore, the use of adult rats as test subjects enables the use of the well-described acute duodenal ulcerogen cysteamine (34, 45).

Verkman's group recently discovered a thiazolidinone compound, named $CFTR_{inh}$-172, that has shown considerable promise as an acute and selective inhibitor of CFTR function. In early studies, they demonstrated a lack of toxicity for whole animal studies combined with a high potency and selectivity for CFTR over other ion channels and transporters as well as inhibition of intestinal secretion (33, 51). This high potency and selectivity enabled the use of $CFTR_{inh}$-172 in rats, as opposed to less potent and more toxic anion channel inhibitors such as NPPB, which are too toxic for whole animal experiments (32). Therefore, to further test our hypothesis that

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diminished DBS due to CFTR dysfunction is associated with protection against acid-related duodenal injury, we examined the effect of $CFTR_{inh}$-172 on DBS and cysteamine-induced duodenal ulceration in rats.

METHODS

Chemicals and animals. $CFTR_{inh}$-172 was synthesized according to the published chemical structure of $CFTR_{inh}$-172 (33) and purified with HPLC, with the structure verified by nuclear magnetic resonance. $CFTR_{inh}$-172 was dissolved with DMSO at 1 mg/10 μl for intraperitoneal injection and 1 mM for duodenal perfusion; the stock solution was kept at −20°C until use. DMSO (1%) in saline was used as the vehicle for intraperitoneal injection. Cysteamine, ethanolamine, and other chemicals were obtained from Sigma (St. Louis, MO). Cysteamine or ethanolamine was dissolved in ethanol and diluted with saline at 50 mg/ml.

All studies were performed with the approval of the Veterans Affairs Institutional Animal Care and Use Committee. Male Sprague-Dawley rats weighing 200–250 g (Harlan; San Diego, CA) were fasted overnight but had free access to water.

Measurement of HCO_3^- secretion. Under isoflurane anesthesia (1.5–2.0%) using a rodent anesthesia inhalation system (Summit Medical Systems; Bend, OR), rats were placed supine on a recirculating heating block system (Summit Medical) to maintain body temperature at 36–37°C, as monitored by a rectal thermistor. Prewarmed saline was infused via the right femoral vein at 1.08 ml/h using a Harvard infusion pump (Harvard Apparatus; Holliston, MA); blood pressure was monitored via a catheter placed in the left femoral artery using a pressure transducer (Kent Scientific; Torrington, CT). DBS was measured with the pH stat method through the duodenal loop as previously described (4, 14). In brief, the stomach and duodenum were exposed, and the forestomach wall was incised using a thermal cautery device (Geiger Medical Technologies; Monarch Beach, CA). A polyethylene tube (5 mm diameter) was inserted through the incision until it was 0.5 cm caudal to the pyloric ring, where it was secured with a nylon ligature. The distal duodenum was ligated proximal to the ligament of Treitz before the duodenal loop was filled with 1 ml saline prewarmed to 37°C. The distal duodenum was then incised, through which another polyethylene tube was inserted and sutured into place. To prevent contamination of the perfusate from bile-pancreatic juice, the pancreaticobiliary duct was ligated just proximal to its insertion into the duodenal wall. The resultant closed proximal duodenal loop (perfused length 2 cm) was perfused with prewarmed saline using a peristaltic pump (Fisher Scientific; Pittsburgh, PA) at 1 ml/min. Input (perfusate) and effluent of the duodenal loop were recirculated through a reservoir in which the perfusate was bubbled with 100% O_2. The pH of the perfusate was kept constant at pH 7.0 with a pH stat (models PHM290 and ABU901, Radiometer Analytical; Lyon, France). Secreted HCO_3^- was calculated from the amount of 0.01 N HCl used for titration and expressed as micromoles per minute per centimeter.

The loop was perfused with saline (pH 7.0) for 30 min, during which DBS gradually declined to a steady state, which we define as "basal DBS." The time (t) was then defined as $t = -5$ min. The duodenal loop was then further perfused with pH 7.0 saline for 15 min (basal, $t = -5$ to 10 min) with pH stat measurement of base secretion. Acid solution (pH 2.2 saline) was perfused with a Harvard infusion pump at 1 ml/min ($t = 10$–20 min) followed by pH 7.0 saline with pH stat measurement ($t = 20$–45 min). O_2 gas-bubbled pH 7.0 saline was recirculated with a peristaltic pump, whereas pH 2.2 saline was perfused via a syringe pump. The duodenal loop solution was gently flushed with 5 ml perfusate to rapidly change the perfusate composition at $t = 10$ and 20 min.

To examine the luminal effect of $CFTR_{inh}$-172 on basal DBS, $CFTR_{inh}$-172 (0.1, 1.0, and 10 μM in final concentration) was applied stepwise every 10 min into the reservoir and circulated through the duodenal loop, because $CFTR_{inh}$-172 has a limited water solubility of ~20 μM (33). The luminal effect of $CFTR_{inh}$-172 on acid-induced DBS was examined with 10 μM $CFTR_{inh}$-172 dissolved in pH 2.2 saline. Furthermore, to examine the effects of the systemic treatment with $CFTR_{inh}$-172 on DBS, $CFTR_{inh}$-172 (1 mg/kg ip) or vehicle was administered 1, 3, or 24 h before the rats were anesthetized. The dose systemically administered in the present study, 1 mg/kg, has been reported to have an antidiarrheal effect (51), and its half-times are 0.14 h for redistribution and 10.3 h for elimination in rats when a single intravenous bolus is infused (44).

Duodenal ulcer induction. Duodenal ulcers were induced by cysteamine using a single subcutaneous injection, as previously described (45). We used ulcerogenic (300 mg/kg) and nonulcerogenic doses (100 mg/kg) of cysteamine. The nonulcerogenic cysteamine-like compound ethanolamine was used in the vehicle control group (2). Rats were given cysteamine or ethanolamine subcutaneously under isoflurane anesthesia (4%) and were allowed to recover and returned to their cages while being closely observed for the first 1 h. Although it has been reported that an ulcerogenic dose of cysteamine in conscious animals induces hypotension and neurological symptoms (7), we did not see any toxic symptoms during the observation period in the present study. To examine the effect of $CFTR_{inh}$-172 on duodenal ulcer formation, $CFTR_{inh}$-172 (1 mg/kg ip) or vehicle was administered 1 h before cysteamine treatment, with the duodenum excised 24 h after cysteamine treatment, because cysteamine induces mucosal injury within 30 min of its administration with frank ulceration developing within 24 h (45). Images of the duodenal mucosa were taken with a digital camera (Nikon; Tokyo, Japan) with the area of ulceration calculated using Adobe Photoshop. We confirmed gross damage scores with damage assessment using routine hematoxylin and eosin (H&E) histological sections. Duodenal tissue was cut into longitudinal strips and fixed with 10% neutral formalin solution. Paraffin-embedded sections were cut and stained with H&E and then observed under a light microscope (Zeiss; Jena, Germany) attached with a charged-coupled device color video camera (Hamamatsu Photonics; Hamamatsu, Japan), and the images were captured with imaging software (Simple PCI, Compix Imaging Systems; Cranberry Township, PA). Ulcer severity was scored using a scale adapted from previously published studies (46) by an independent outside observer who was blinded to the treatments given to each animal, using a scale of 0–3, where *0* is no ulcer, *1* is a superficial ulcer (mucosal erosion), *2* is a deep ulcer (beyond submucosa or involving the muscularis propria), and *3* is a perforating or penetrated ulcer. The maximum score was *3*. Because the ulcers were sometimes paired on opposite wall of the duodenum, i.e., kissing ulcer, the area was the addition of the two ulcers, whereas the score was the mean of the two ulcer severity scores (46).

Gastric acid and HCO_3^- secretion. Because one of the mechanisms by which cysteamine induces duodenal mucosal injury is through the stimulation of gastric acid secretion with concurrent inhibition of DBS, which lowers duodenal pH (30), we sought to determine whether $CFTR_{inh}$-172 affected gastric acid secretion. Furthermore, gastric acid and HCO_3^- secretion rates have not previously been measured in the presence of CFTR dysfunction.

We thus measured gastric acid and HCO_3^- secretion in the presence of vehicle or $CFTR_{inh}$-172 pretreatment 1 h before anesthesia induction using previously described methodology (3, 10). A 5-mm-diameter polyvinyl tube was inserted through a forestomach incision made using a thermal cautery, which was secured with a nylon suture. The effluent tube was inserted through the proximal duodenum and secured at the pyloric ring. The stomach was rinsed with prewarmed saline and perfused at 1 ml/min with a Harvard infusion pump. To prevent the gastric content from refluxing into the esophagus, the lower esophagus was gently ligated with nylon sutures without interfering with the vagi- and paraesophgeal vasculature, as previously described (49). Effluent pH and [CO_2] were continuously measured with a modification of previously published methods (10) with pH and

CO_2 electrodes, respectively, placed in series using flow-through cells (Micro Flow-Through pH and CO_2 electrodes, Lazar Research Laboratories; Los Angeles, CA). The effluent was collected every 10 min, with acidity measured by back-titration with 0.1 N NaOH using an Autoburette (Radiometer; Copenhagen, Denmark). Gastric acid output was expressed as milliequivalents per 10 min. Total [CO_2] was calculated from pH and [CO_2] using the Henderson-Hasselbalch formula as previously described (3) and expressed as total CO_2 output (in μmol/min). After a 30-min stabilization, time was set as $t = 0$. The basal acid secretion was measured for 30 min (t = 0–30 min), followed by a stimulated secretion with a submaximal dose of pentagastrin (16 $\mu g \cdot kg^{-1} \cdot h^{-1}$ continuous iv) for 60 min (t = 30–90 min) as previously described (48).

Statistics. All data are expressed as means ± SE of 6 rats/group. Comparisons between groups were made by one-way ANOVA, followed by Fischer's least-significant difference test. Comparisons of two groups were assessed by unpaired, one-tailed *t*-test. Nonparametric injury data were analyzed using the Mann-Whitney test. *P* values of 0.05 were taken as significant.

RESULTS

Effects of $CFTR_{inh}$-172 perfusion on basal and acid-induced DBS. Because the CFTR is present in the apical membrane of duodenal epithelial cells (9), we examined the effect of luminal application of $CFTR_{inh}$-172 on basal and acid-induced DBS. $CFTR_{inh}$-172 (0.1–10 μM) did not affect basal DBS (Fig. 1*A*). DBS was increased after acid exposure, whereas luminal perfusion with 10 μM $CFTR_{inh}$-172 (dissolved in pH 2.2 saline) abolished acid-induced increases of DBS (Fig. 1*B*), indicating that luminal $CFTR_{inh}$-172 inhibited acid-induced but not basal DBS, suggesting that its effect is limited to acid-activated CFTR function.

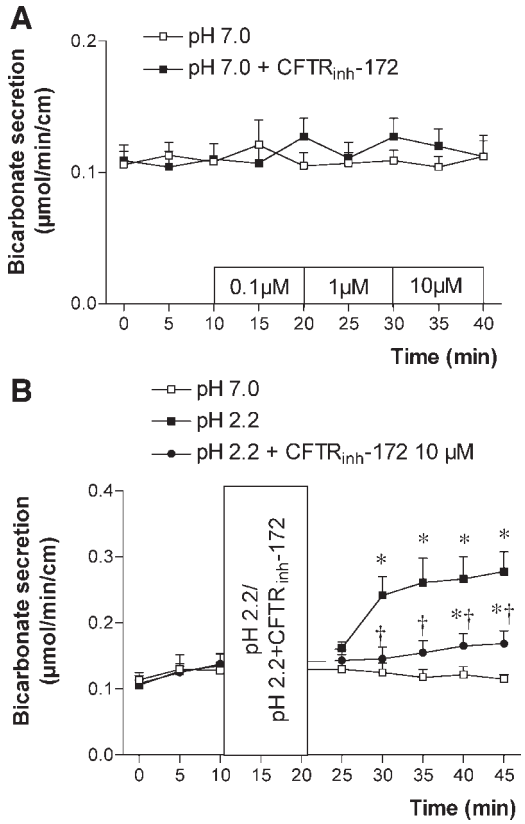

Fig. 1. Effects of cystic fibrosis transmembrane conductance regulator inhibitor $CFTR_{inh}$-172 on basal and acid-induced duodenal bicarbonate (HCO_3^-) secretion (DBS) in rats. *A*: $CFTR_{inh}$-172 (0.1–10 μM) had no effect on basal DBS. *B*: luminally applied $CFTR_{inh}$-172 (10 μM) inhibited the acid-induced increase of DBS. Data are expressed as means ± SE; $n = 6$. *$P < 0.05$ vs. the pH 7.0 group; †$P < 0.05$ vs. the pH 2.2 group.

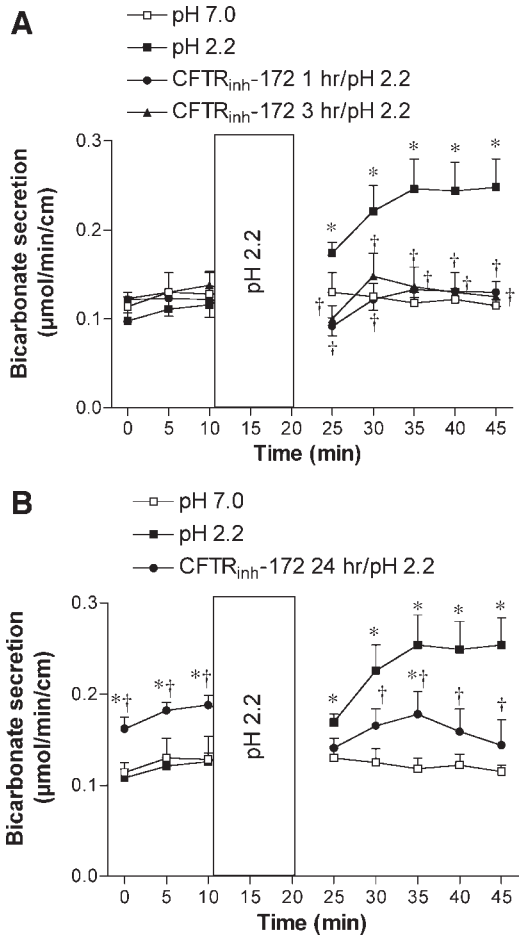

Fig. 2. Effects of the short- and long-term $CFTR_{inh}$-172 administration on acid-induced DBS in rats. *A*: 1- or 3-h pretreatment with $CFTR_{inh}$-172 (1 mg/kg ip) inhibited acid-induced DBS, whereas no effect was observed on basal DBS. Note that the pH 2.2 group was pretreated with vehicle 1 h before the experiments. Data are expressed as means ± SE; $n = 6$. *$P < 0.05$ vs. the pH 7.0 group; †$P < 0.05$ vs. the pH 2.2 group. *B*: acid-induced DBS was inhibited by $CFTR_{inh}$-172 (1 mg/kg ip) pretreatment 24 h before DBS measurement. Note that basal DBS was enhanced in the $CFTR_{inh}$-172 24-h group. Note that the pH 2.2 group was pretreated with vehicle 24 h before the experiments. Data are expressed as means ± SE; $n = 6$. *$P < 0.05$ vs. the pH 7.0 group; †$P < 0.05$ vs. the pH 2.2 group.

Effects of $CFTR_{inh}$-172 pretreatment on basal and acid-induced DBS. Acid-induced DBS was abolished 1 or 3 h after $CFTR_{inh}$-172 treatment, whereas basal DBS was unchanged (Fig. 2*A*), confirming the acute inhibitory effect of $CFTR_{inh}$-172 on DBS, similar to the effect of the compound on intestinal anion secretion (51). Basal DBS was increased 24 h after $CFTR_{inh}$-172 treatment, whereas acid-induced DBS was still reduced (Fig. 2*B*), indicating that the inhibition of acid-induced DBS with $CFTR_{inh}$-172 lasted at least 24 h.

Effects of $CFTR_{inh}$-172 pretreatment on cysteamine-induced duodenal ulceration. Duodenal ulceration was observed 24 h after treatment with an ulcerogenic dose of cysteamine (300 mg/kg; Fig. 3*A*), which was not observed with a nonulcero-

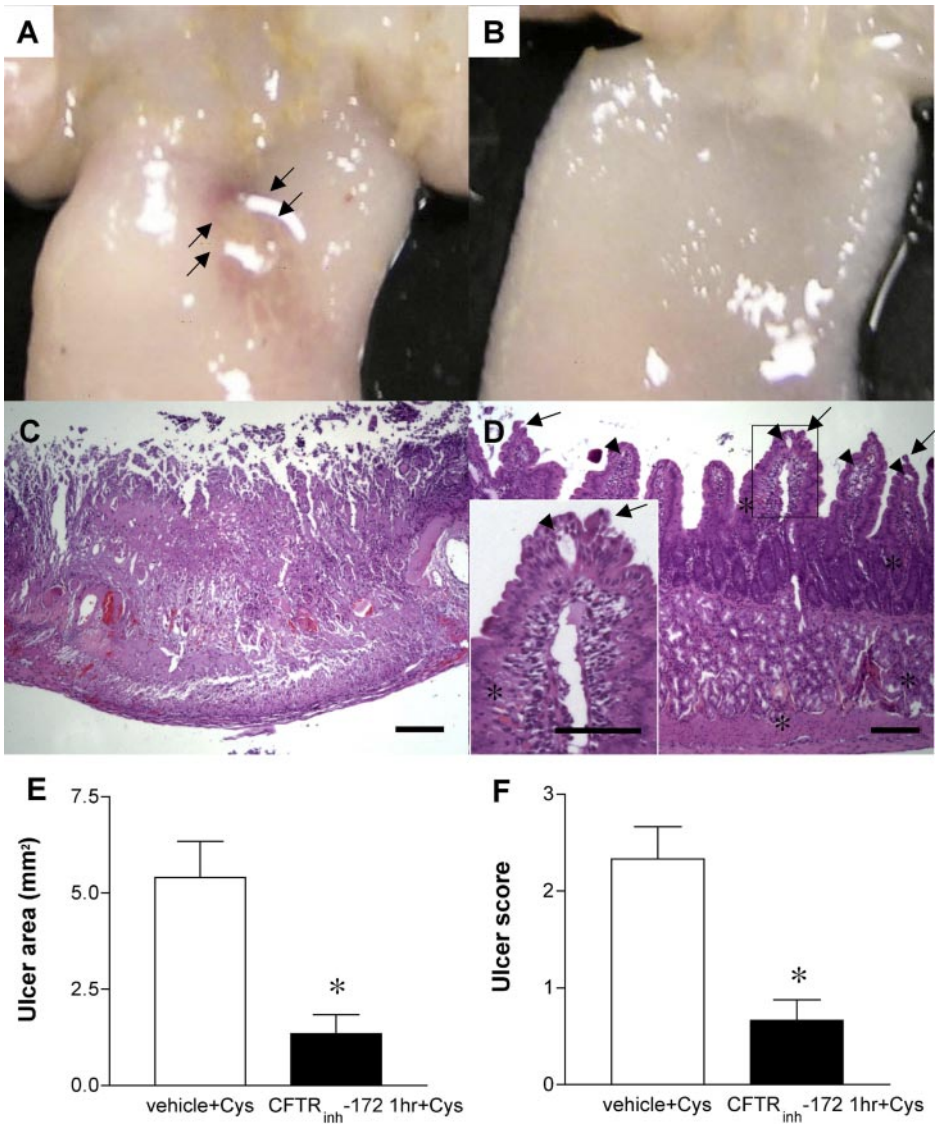

Fig. 3. Effects of $CFTR_{inh}$-172 pretreatment on duodenal ulcer formation induced by cysteamine (Cys) in rats. $CFTR_{inh}$-172 (1 mg/kg ip) was given 1 h before Cys treatment. *A*: a round, deep duodenal ulcer (arrows) was clearly observed 24 h after Cys treatment *B*: no ulcer was recognized in the duodenum with $CFTR_{inh}$-172 pretreatment. *C*: hematoxylin and eosin (H&E)-stained sections (original magnification ×100; reference bar = 100 μm) of Cys-induced duodenal ulcer. Transmural hemorrhage, inflammation, and massive necrosis filling the ulcer bed were seen. *D*: H&E-stained section of the duodenum of $CFTR_{inh}$-172-pretreated rats. No apparent injury was seen. Note that there are sloughed epithelial cells (arrows) in some villous tips, subepithelial edema (arrowheads) in some villi, and hemorrhage (*) in the villi (*inset*: original magnification ×200) and submucosa. *E*: Cys-induced duodenal ulcer formation was attenuated by $CFTR_{inh}$-172 pretreatment ($CFTR_{inh}$-172 1 h + Cys). Data are expressed as means ± SE; $n = 6$.*$P < 0.05$ vs. the vehicle + Cys group. *F*: quantitation of ulcer severity (scores of 0–3). Data are expressed as means ± SE; $n = 6$. *$P < 0.05$ vs. the vehicle + Cys group.

genic dose of cysteamine (100 mg/kg) or ethanolamine treatment (data not shown). Two of the six ulcers were kissing ulcers. Pretreatment with $CFTR_{inh}$-172 1 h before cysteamine treatment reduced duodenal ulcer formation, as assessed at 24 h after cysteamine treatment (Fig. 3*B*). There were no kissing or multiple ulcers observed in the $CFTR_{inh}$-172-pretreated group. This macroscopic observation was confirmed in the histological H&E-stained sections, where transmural ulceration with massive necrosis was observed in the duodena of cysteamine-treated rats (Fig. 3*C*). Occasional mucosal erosions, but no deep ulcers, were observed in the duodena of $CFTR_{inh}$-172-pretreated rats (Fig. 3*D*). As shown in Fig. 3*D*, for example, no erosions were observed, but there were a few sloughed epithelial cells, subepithelial edema in some villi, and minimal hemorrhage in the villous mucosa (lamina propria mucosa) and in the submucosa. The ulcer area was significantly attenuated by $CFTR_{inh}$-172 pretreatment 1 h before cysteamine treatment (Fig. 3*E*). The ulcer score was also reduced by $CFTR_{inh}$-172 pretreatment (Fig. 3*F*).

Effects of $CFTR_{inh}$-172 pretreatment on gastric acid and HCO_3^- secretion. $CFTR_{inh}$-172 pretreatment had no effect on either basal or pentagastrin-induced acid secretion in the stomach (Fig. 4*A*). Furthermore, there was no difference in total gastric CO_2 output between vehicle- or $CFTR_{inh}$-172-treated groups (Fig. 4*B*), suggesting that the CFTR does not directly mediate gastric acid or HCO_3^- secretion.

DISCUSSION

We demonstrated that $CFTR_{inh}$-172 acutely inhibited stimulated duodenal CFTR function, as measured as acid-induced DBS, and attenuated cysteamine-induced duodenal ulceration. Furthermore, regardless of the effect of $CFTR_{inh}$-172 on gastric acid and DBS, CFTR inhibition protected the duodenal mucosa from cysteamine-induced acid-related injury, supporting our hypothesis that DBS and injury do not always correlate. These studies further suggest that CFTR dysfunction, with a consequent impairment of stimulated DBS, does not increase the susceptibility of the duodenal mucosa to acid-induced injury.

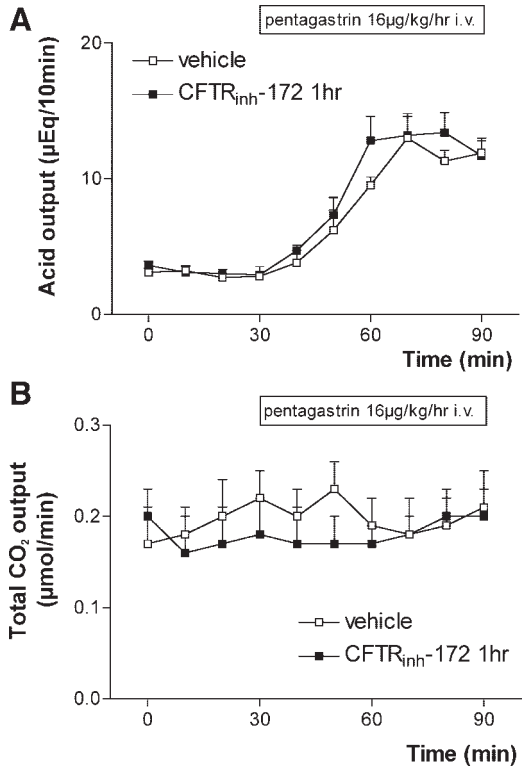

Fig. 4. Effects of 1-h $CFTR_{inh}$-172 pretreatment on gastric acid and CO_2 output in rats. $CFTR_{inh}$-172 (1 mg/kg ip) had no effect on basal and pentagastrin-stimulated gastric acid (*A*) or CO_2 output (*B*) compared with vehicle control. Data are expressed as means ± SE; $n = 6$.

Our study is the first to examine the effect of $CFTR_{inh}$-172 on DBS. CFTR inhibition with a single intraperitoneal dose of $CFTR_{inh}$-172 was observed at least 1 h after its administration and lasted at least 24 h thereafter, consistent with earlier studies by Verkman's group, although they showed the inhibition only up to 6 h in a enterotoxin-induced intestinal diarrhea model (33, 44, 51). Verkman's group has also shown that $CFTR_{inh}$-172 has measurable concentrations in the small intestine 60 min after an intravenous injection (44). The inhibitor did not affect basal DBS but rather only inhibited augmented DBS in response to luminal acid challenge. This result is discordant with those observed in vivo in murine models of genetic CFTR dysfunction and in biopsies obtained from patients with CF (8, 18, 19, 39). This discrepancy may reflect the induction of acute rather than chronic CF dysfunction. Also likely is that $CFTR_{inh}$-172 only inhibited activated CFTR, because $CFTR_{inh}$-172 only inhibits activated and not basal CFTR function in a variety of tissues and species (33, 43, 51, 52). $CFTR_{inh}$-172 most likely inhibits CFTR by binding to the cytoplasmic loops of the cAMP-activated protein (33, 43, 44). This would explain our observation that systemically applied $CFTR_{inh}$-172 diminished the acid-induced DBS increase, suggesting that its cytoplasmic binding site is related to CFTR activation, such as by protein kinase phosphorylation (21).

Our interest in CF in the context of duodenal injury originated in the clinical observation that CF patients, despite low DBS and rates of high acid secretion, have an unexpectedly low prevalence of duodenal ulceration (6, 15, 29, 31, 40), which we term the "CF paradox" (24). In our studies of DBS, we used genetic and pharmacological models of CFTR dysfunction to study duodenal defense mechanisms. We inhibited CFTR function with nonselective anion inhibitors such as NPPB (4) and measured HCO_3^- secretion and mucosal injury in CFTR mutant mice (17). Nevertheless, the acid-induced injury we previously assessed was early and superficial, as detected by propidium iodide, and not likely fully comparable to deep injury expressed clinically as ulceration. Furthermore, genetic CFTR mutations may be compensated during development, perhaps confounding the effect of CFTR dysfunction.

The most accepted rodent duodenal ulcer model is the cysteamine rat model, which has the advantages of rapid and reproducible formation of duodenal ulcers by a mechanism thought to involve a diminution of duodenal host defenses to luminal acid, such as DBS, mucus secretion, and mucosal blood flow, and enhancement of aggressive factors such as acid and pepsin secretion (20, 27, 50). Other hypotheses regarding the mechanism of action of cysteamine include somatostatin depletion (47) and alteration of the mucosal redox state (25). Despite the increased acid secretion observed in cysteamine-treated rats, similar duodenal ulceration was not observed in other models of acid hypersecretion, such as pentagastrin-treated rats, strongly suggesting that a decrease of mucosal protective factors underlies the primary ulcerogenic effect of cysteamine treatment (27, 30, 35). Bridén et al. (7) have suggested that cysteamine impairs the augmentation of HCO_3^- secretion in response to luminal acid while not affecting prostaglandin-stimulated DBS, consistent with an impairment of duodenal acid-sensing mechanisms. Cysteamine rapidly induces duodenal epithelial injury, with changes detectable 30 min after administration (50), accompanied by increased gastric acid output (27) and decreased duodenal blood flow (1, 50), with ulcers appearing within 24 h (30, 45). The time course of cysteamine-induced ulcer induction fortuitously matched well with the duration of inhibition of DBS by $CFTR_{inh}$-172. Interestingly, enhanced basal DBS was observed 24 h after $CFTR_{inh}$-172 treatment, suggesting that CFTR inhibition may upregulate the basal HCO_3^- secretory pathway, which appears to be mediated by a mechanism distinct from the acid-stimulated pathway (22). We speculate that $CFTR_{inh}$-172 impairs cysteamine-induced ulceration by limiting the HCO_3^- exit from epithelial cells, increasing the overall cellular buffer capacity and thus limiting cellular acidification during luminal acid stress.

Whether CFTR inhibition affects any defence mechanism other than DBS, such as blood flow or mucus secretion, is still unknown. Histological sections revealed that cysteamine-induced ulceration was inhibited with $CFTR_{inh}$-172 pretreatment, whereas minimum injury such as epithelial cell sloughing, subepithelial edema, and mild duodenitis was present, suggesting that the protective effects of $CFTR_{inh}$-172 mostly affected epithelial cells, with lesser effects on the subepithelial mucosa. Because cysteamine increases gastric acid output and decreases DBS and Brunner's gland secretions (7, 16, 26, 38), $CFTR_{inh}$-172 might selectively protect epithelial cells from luminal injurious factors while having lesser effects on submucosal factors such as decreased blood flow (50), reducing injury depth.

In conclusion, $CFTR_{inh}$-172 is a useful tool to produce acute CFTR dysfunction in rodents as a CF model and also to examine the role of CFTR in models of up to 24-h duration.

CFTR inhibition reduces acid-induced DBS but attenuates duodenal ulcer formation, supporting our hypothesis that CFTR dysfunction is associated with protection of the duodenal mucosa from acid-related injury.

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