Extracellular UTP stimulates electrogenic bicarbonate secretion across CFTR knockout gallbladder epithelium

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Clarke, Lane L., Matthew C. Harline, Lara R. Gawenis, Nancy M. Walker, John T. Turner, and Gary A. Weisman. Extracellular UTP stimulates electrogenic bicarbonate secretion across CFTR knockout gallbladder epithelium. Am J Physiol Gastrointest Liver Physiol 279: G132–G138, 2000.—The loss of cystic fibrosis transmembrane conductance regulator (CFTR)-mediated transepithelial HCO3− secretion contributes to the pathogenesis of pancreatic and biliary disease in cystic fibrosis (CF) patients. Recent studies have investigated P2Y2 nucleotide receptor agonists, e.g., UTP, as a means to bypass the CFTR defect by stimulating Ca2+-activated Cl− secretion. However, the value of this treatment in facilitating transepithelial HCO3− secretion is unknown. Gallbladder mucosae from CFTR knockout mice were used to isolate the Ca2+-dependent anion conductance during activation of luminal P2Y2 receptors. In Ussing chamber studies, UTP stimulated a transient peak in short-circuit current (Isc) that declined to a stable plateau phase lasting 30–60 min. The plateau Isc after UTP was Cl− independent, HCO3− dependent, insensitive to bumetanide, and blocked by luminal DIDS. In pH stat studies, luminal UTP increased both Isc and serosal-to-mucosal HCO3− flux (Jsc-HCO3−) during a 30-min period. Substitution of Cl− with gluconate in the luminal bath to inhibit Cl−/HCO3− exchange did not prevent the increase in Jsc-m and Isc during UTP. In contrast, luminal DIDS completely inhibited UTP-stimulated increases in Jsc-m and Isc. We conclude that P2Y2 receptor activation results in a sustained (30–60 min) increase in electrogenic HCO3− secretion that is mediated via an intracellular Ca2+-dependent anion conductance in CF gallbladder.

cystic fibrosis; biliary system; P2Y2 receptor; nucleotide receptor; purinoceptor; chloride

Cystic fibrosis (CF) disease is caused by mutations in the gene encoding the CF transmembrane conductance regulator (CFTR) protein, a cyclic nucleotide-activated anion channel (3, 5). CFTR mutations result in at least two abnormalities of transepithelial electrolyte and water transport that contribute to the pathogenesis of CF. First, it is well documented that a deficiency in cAMP-dependent regulation of transepithelial salt and water secretion occurs in CF and may lead to the dehydration of luminal mucus and debris (37). Second, it is also recognized that abnormal transepithelial pH regulation occurs in CF epithelia (19, 32). Measurements of ionic currents across airway and intestinal epithelia (16, 34), as well as reports of deficient alkalization of pancreatic juice, biliary secretions, and the duodenal lumen, indicate a loss of HCO3− secretion in CF (for review, see Refs. 11, 13, 19, 32). This deficiency likely relates to the role that CFTR plays in transepithelial HCO3− secretion and the possibility that CFTR can function as a cAMP-activated HCO3− channel (9, 18, 20, 31).

In recent years, it has been proposed that extracellular nucleotide (ATP, UTP) therapy may be useful in the symptomatic treatment of CF. In a number of CF epithelial tissues (6, 10, 14, 23, 25), topically applied UTP binds the P2Y2 nucleotide receptor, resulting in the activation of alternative Cl− conductances (e.g., CLCA) primarily via the phospholipase C/inositol 1,4,5-trisphosphate/intracellular Ca2+ (Ca2+i) signaling pathway. Thus deficient transepithelial salt and water secretion in CF may be partially reversed through activation of Cl− secretion by a pathway that bypasses the CFTR. Because the alternative conductances are anion selective, we hypothesized that stimulation of the P2Y2 receptor may also result in transepithelial HCO3− secretion and therefore be useful in treating abnormal transepithelial pH regulation in CF.

Previous studies (6, 10, 33) have linked the expression of the P2Y2 receptor with the nucleotide-dependent stimulation of Ca2+-activated anion secretion in murine, rat, and human biliary epithelia. However, mRNA expression studies (29, 36) have also shown that the CFTR is abundant in the gallbladder epithelia of both humans and mice. Functional studies verify the role of the CFTR in cAMP-stimulated transepithelial anion secretion in these tissues, and, recently, it was shown (24) that the CFTR primarily mediates electrogenic HCO3− secretion rather than Cl− secretion across the murine gallbladder. Therefore, to isolate the effect of UTP on Ca2+-mediated HCO3− secretion and to simulate the CF condition, studies using the pH stat tech-
tique were performed with freshly excised gallbladders from CFTR knockout mice.

MATERIAL AND METHODS

Animals. Weanling mice (2–4 mo of age) born to animals heterozygous for the disrupted murine homologue of the cfr gene (B6.129-Cfrtm1Unc, C57BL/6J-Cfrtm1Unc) were used. The genotype of each littermate was determined using a PCR technique employing primers specific for murine cfr and the neomycin resistance/cfr junction, as previously described (7). CFTR(−/−) and CFTR(+/+) mice were homozygous for the disrupted cfr gene and the wild-type cfr gene, respectively. The mice were fed standard laboratory mouse chow and water ad libitum until the evening before an experiment and then only drinking water was provided. The drinking water for all mice contained an osmotic laxative (polyethylene glycol) to prevent intestinal impaction in the CFTR(−/−) mice (7). The University of Missouri-Columbia Institutional Animal Care and Use Committee approved all experiments involving the animals.

In vitro bioelectric and pH stat measurements. Mice were killed on the day of the experiment by brief exposure to an atmosphere of 100% CO₂ to induce basal narcosis, followed by a surgically induced bilateral pneumothorax. The gallbladder and a portion of surrounding liver were excised en masse via an abdominal incision and immediately placed in ice-cold, oxygenated Ringer solution (containing 1 μM indo- methacin to prevent prostanoid generation). Under a dissecting microscope, the gallbladder was dissected free of hepatic tissue, opened longitudinally, and placed mucosal-side up on coarse-gauge nylon mesh. The gallbladder on nylon mesh was mounted horizontally in a modified Ussing chamber (10). The serosal bath was replaced with an equimolar concentration of 3 mM CaSO₄ was added to overcome Ca²⁺ chelation. The solutions were circulated throughout the experiment by gas lift and warmed to 37°C by water-jacketed reservoirs. Before each experimental protocol, gallbladders were equilibrated for 20 min under short-circuited conditions with TTX (0.1 μM) in the serosal bath to minimize variation due to intrinsic neural tone.

Transepithelial short-circuit current (Iₛ𝑐, in μeq⋅cm⁻²⋅h⁻¹) was measured using an automatic voltage clamp (VCC-600, Physiologic Instruments, San Diego, CA) and calomel electrodes that were connected to the chambers by 4% agar-3 M KCl bridges, as previously described (9). Iₛ𝑐 and automatic fluid resistance compensation current were applied through Ag-AgCl electrodes connected to the chamber baths via 4% agar-NaCl bridges. Every 5 min during an experiment, a 5-mV pulse was passed across the gallbladder tissue to determine the total tissue conductance (Gₜ, mS/cm² tissue surface area) by measuring the magnitude of the resulting current deflections and applying Ohm’s law. The serosal bath served as ground in all experiments.

The serosal-to-mucosal flux of HCO₃⁻ (Jₛ-m, in μeq⋅cm⁻²⋅h⁻¹) was measured by pH stat titration of the luminal bath (4 ml) to pH 7.4 using 5 mM HCl delivered by either a computer-aided titrimeter (Fisher, model 455/465) or manual addition of titrant. The volume of added acid was used to calculate the HCO₃⁻ (base) flux, taking into account the time period and the surface area of the tissue. Typically, Jₛ-m stabilized within 30 min after the tissue was mounted, and the luminal solution was replaced to refresh transepithelial ion gradients and remove secreted mucus. A 30-min basal flux period was initiated, and then UTP (100 μM) was added to the luminal bath. Iₛ𝑐 assumed a plateau phase after 5 min, and a second 30-min flux period was initiated.

Statistics. Student’s paired or unpaired t-tests were used for statistical comparisons. P ≤ 0.05 was considered statistically significant. Unless otherwise indicated, data are presented as means ± SE.

Materials. Immediately before each experiment, a stock solution of 10 mM UTP (Boehringer-Mannheim, Indianapolis, IN) was made in NaCl and titrated to pH 7.4. Indomethacin (Sigma Chemical, St. Louis, MO) was dissolved in DMSO at a stock concentration of 0.01 M. DIDS (Aldrich Chemical, Milwaukee, WI) was dissolved in NaCl solution at a stock concentration of 0.03 M. TTX (Sigma Chemical) was dissolved in 0.2% acetic acid at a stock concentration of 0.0001 M. All other reagents were obtained from either Sigma Chemical, Aldrich Chemical, or Fisher Scientific (Springfield, NJ).

RESULTS

Ussing chamber studies. Previous studies (10) of murine gallbladder epithelium established the presence of a luminal membrane P₂Y₁ receptor that is stimulated by UTP and results in Ca²⁺-activated, transepithelial anion secretion. In normal murine gallbladder, CFTR is a basally active anion conductance that has been shown to mediate both electrogenic Cl⁻ and HCO₃⁻ secretion (24). Because the presence of CFTR complicates the analysis of Ca²⁺-activated anion currents, studies evaluating the ionic basis of the UTP-stimulated Iₛ𝑐 were performed on freshly excised gallbladder epithelium from CFTR knockout mice. As shown in Fig. 1, cAMP-dependent stimulation of CFTR(+/+) gallbladders by a forskolin treatment induced a large Iₛ𝑐 response, whereas treatment of the CFTR(−/−) gallbladders was essentially without effect. In contrast, UTP treatment of the luminal membrane stimulated a nearly equivalent Iₛ𝑐 response in both CFTR(+/+) and CFTR(−/−) gallbladders. Previous Ussing chamber studies of murine gallbladder have established that 100 μM UTP induces a near-maximal Iₛ𝑐 response when added to the luminal bath but is relatively ineffective when added to the serosal bath (10).

An Iₛ𝑐 recording trace of the UTP response in a CFTR(−/−) gallbladder epithelium with NaCl in the luminal bath and KRB in the serosal bath is shown in Fig. 2A. Typical of Ca²⁺-activated transepithelial anion secretion, the Iₛ𝑐 after UTP treatment immediately increased to a maximum value and then decreased within minutes to a stable plateau where it remained elevated over baseline. The plateau Iₛ𝑐 in the UTP-treated CFTR(−/−) gallbladder typically was elevated for over 30 min. Subsequent treatment of the gallblad-
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Fig. 1. Comparison of the maximal change in short-circuit current ($I_{sc}$) after treatment with either 10 \(\mu\text{M}\) forskolin (Forsk) or 100 \(\mu\text{M}\) UTP in gallbladder epithelium from cystic fibrosis transmembrane conductance regulator (CFTR(+/+) and CFTR(−/−)) mice (\(n=5\) each). $\Delta I_{sc}$ has been normalized to 1 cm\(^2\) of mucosal surface area. *Significantly different from CFTR(+/+)

with bumetanide, an inhibitor of the Na\textsuperscript{+}-K\textsuperscript{+}-2Cl\textsuperscript{−} cotransporter, did not affect the $I_{sc}$ suggesting that the plateau $I_{sc}$ was not due to Cl\textsuperscript{−} secretion. To evaluate the Cl\textsuperscript{−} dependence of the UTP-induced $I_{sc}$ response, CFTR(−/−) gallbladders were bathed in identical solutions except that Cl\textsuperscript{−} was replaced with gluconate\textsuperscript{−}. As shown in Fig. 2B, the time course of the immediate $I_{sc}$ response to UTP was reduced in the Cl\textsuperscript{−}-free Ringer solution, whereas the plateau $I_{sc}$ phase was not affected. These findings are consistent with the hypothesis that the plateau phase of the UTP-induced $I_{sc}$ response in CFTR(−/−) gallbladder epithelium represents electrogenic HCO\textsubscript{3}\textsuperscript{-} secretion. To evaluate the HCO\textsubscript{3}\textsuperscript{-} dependence of the sustained $I_{sc}$ response, CFTR(−/−) gallbladders were bathed in HCO\textsubscript{3}\textsuperscript{-}-free Ringer solution (TES buffered, gassed with 100% O\textsubscript{2}) and treated with 100 \(\mu\text{M}\) methazolamide (luminal + serosal) to inhibit endogenous HCO\textsubscript{3}\textsuperscript{-} production (Fig. 2C). This condition markedly attenuated the initial $I_{sc}$ response and abolished the $I_{sc}$ plateau after UTP treatment. Next, we asked whether the plateau $I_{sc}$ after UTP could be inhibited at the luminal membrane with DIDS (300 \(\mu\text{M}\)), a blocker of the Ca\textsuperscript{2+}-activated anion conductance (4, 8). As shown in Fig. 2D, DIDS treatment completely abolished the plateau $I_{sc}$ response after UTP addition. DIDS at 100 \(\mu\text{M}\) concentration also completely inhibited the plateau phase of the UTP-induced $I_{sc}$ response (data not shown). As a control, 300 \(\mu\text{M}\) DIDS was added to the serosal bath after UTP and did not decrease but, unexpectedly, caused an increase in the $I_{sc}$ (+2.3 and +1.9 \(\mu\text{A}\), \(n=2\)).

**pH stat studies.** The preceding findings were consistent with the hypothesis that the plateau phase of the $I_{sc}$ response after UTP treatment represents electrogenic HCO\textsubscript{3}\textsuperscript{-} secretion via a Ca\textsuperscript{2+}-activated anion conductance in murine gallbladder. Therefore, the $I_{sc}$ and $J_{s-m}$ were simultaneously measured using the pH stat technique (both parameters are expressed in \(\mu\text{equiv} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}\) for comparison). The flux studies performed on intact CFTR(−/−) gallbladders consisted of two periods, a 30-min basal flux period followed by addition of luminal UTP and a 30-min treatment flux period (i.e., during the plateau phase of the $I_{sc}$ response to luminal UTP). As shown in Fig. 3, the baseline $J_{s-m}$ across CFTR(−/−) gallbladder was 4.31 ± 0.64 \(\mu\text{equiv} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}\) and the $I_{sc}$ was 2.04 ± 0.28 \(\mu\text{equiv} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}\). During the plateau phase beginning 5 min after luminal UTP addition, the $J_{s-m}$ increased by 0.84 ± 0.21 \(\mu\text{equiv} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}\), which was nearly equivalent to the stable increase in $I_{sc}$ of 0.79 ± 0.32 \(\mu\text{equiv} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}\). Luminal UTP addition did not alter the $G_{t}$ between the two flux periods, indicating that changes in the conductance of the paracellular pathway did not contribute to the increased rate of HCO\textsubscript{3}\textsuperscript{-} secretion (basal $G_t$ = 44.4 ± 5.3; UTP $G_t$ = 47.0 ± 6.1 mS/cm\(^2\)). To evaluate whether direct manipulation of Ca\textsuperscript{2+} mobilization would generate a response similar to that of UTP, additional experiments were performed using the Ca\textsuperscript{2+} ionophore ionomycin (1 \(\mu\text{M}\)) added to the luminal bath of CFTR(−/−) gallbladders. Ionomycin treatment initially caused a rapid $J_{s-m}$ and $I_{sc}$ response similar to UTP, but the plateau phase only lasted ~20 min. However, both $J_{s-m}$ and $I_{sc}$ were significantly increased during the 20-min period after ionomycin treatment ($\Delta J_{s-m} = 1.1 ± 0.3 \mu\text{equiv} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$, \(P<0.05\); peak $\Delta I_{sc}$ after ionomycin = 1.7 ± 0.6 \(\mu\text{equiv} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}\), \(P<0.05\); $\Delta I_{sc}$ 20 min after ionomycin = 0.4 ± 0.2 \(\mu\text{equiv} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}\), not significant; \(n=4\)).

Previous studies have provided evidence that cAMP-dependent stimulation of normal murine gallbladder induces electrogenic HCO\textsubscript{3}\textsuperscript{-} secretion via a CFTR-dependent process (24). For purposes of comparison with the UTP response, we measured the effect of cAMP-dependent stimulation on $J_{s-m}$ and $I_{sc}$ in intact CFTR(+/+) gallbladders. Using forskolin (10 \(\mu\text{M}\)) treatment to increase intracellular cAMP, the $\Delta J_{s-m}$ and $\Delta I_{sc}$ were 1.76 ± 0.42 and 1.98 ± 0.47 \(\mu\text{equiv} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}\), respectively, over basal values (basal $J_{s-m}$ and $I_{sc}$ were 5.18 ± 0.66 and 1.41 ± 0.27 \(\mu\text{equiv} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}\), respectively; \(n=6\)). Thus the plateau phase of the response to UTP treatment in a CFTR(−/−) gallbladder yields a $\Delta J_{s-m}$ that is 48% and a $\Delta I_{sc}$ that is 40% of the responses to forskolin treatment in CFTR(+/+) gallbladder.

The nearly equivalent increases in $\Delta J_{s-m}$ and $I_{sc}$ after UTP treatment suggest a process of electrogenic HCO\textsubscript{3}\textsuperscript{-} secretion mediated by a Ca\textsuperscript{2+}-activated HCO\textsubscript{3}\textsuperscript{-} conductance. However, it is also possible that UTP stimulates a Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} conductance that increases the activity of a luminal membrane Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{-} antiporter system (28). To investigate this possibility, CFTR(−/−) gallbladders were bathed with a Cl\textsuperscript{−}-free solution in the luminal bath to inhibit activity of luminal membrane Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{-} exchange. As shown in Fig. 4, substitution of luminal Cl\textsuperscript{−} with gluconate greatly
reduced the basal $J_{\text{s-m}}$, indicating that Cl\textsuperscript{-}/HCO\textsubscript{3}\textsuperscript{-} exchange contributes significantly to basal HCO\textsubscript{3}\textsuperscript{-} secretion. The basal $I_{\text{sc}}$ in the absence of luminal Cl\textsuperscript{-} was reversed in polarity, largely as a result of uncompensated junction potentials. Nevertheless, UTP treatment in the absence of luminal Cl\textsuperscript{-} stimulated increases in $J_{\text{s-m}}$ and $I_{\text{sc}}$ that were similar to the UTP responses in luminal NaCl solution ($\Delta J_{\text{s-m}} = 1.13 \pm 0.23 \mu\text{eq}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$; $\Delta I_{\text{sc}} = 1.34 \pm 0.35 \mu\text{eq}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$). UTP treatment under these conditions had no effect on $G_t$ (basal $G_t = 19.8 \pm 3.1 \text{mS/cm}^2$; UTP $G_t = 18.7 \pm 2.7 \text{mS/cm}^2$).

We observed that treatment with luminal DIDS abolishes the plateau phase of the $I_{\text{sc}}$ response (Fig. 2), consistent with inhibition of the Ca\textsuperscript{2+}-activated anion conductance (4). Therefore, we asked whether luminal DIDS treatment would inhibit UTP-dependent stimulation of both $J_{\text{s-m}}$ and $I_{\text{sc}}$ during the plateau phase of the response. As shown in Fig. 5, luminal treatment with DIDS (300 $\mu$M) during the plateau phase of the
UTP response eliminated both the increases in $J_{s\rightarrow m}$ and $I_{sc}$ in the CFTR(−/−) gallbladder. The $G_t$ was not different between the basal and UTP treatment periods under these conditions (basal $G_t = 30.1 \pm 5.7$ mS/cm², UTP $G_t = 29.0 \pm 3.4$ mS/cm²). The ability of luminal DIDS to block both the $I_{sc}$ and $J_{s\rightarrow m}$ is consistent with the conclusion that $\text{Ca}^{2+}$-activated anion channels mediate electrogenic $\text{HCO}_3^-$ secretion across the murine CF gallbladder epithelium.

**DISCUSSION**

Loss of functional CFTR activity in the epithelia of CF patients results not only in a deficit of salt and water secretion, but also severely limits regulated $\text{HCO}_3^-$ permeation across epithelia. The adverse effects of this deficit likely contribute to the pathological changes in the pancreatic and biliary ducts of CF patients (29, 33). Previously, it has been proposed (21, 22) that topical UTP therapy directed at luminal P2Y$_2$ receptors in CF epithelia could restore salt and water secretion via the activation of a $\text{Ca}^{2+}$-activated anion conductance. On the basis of our present results, we propose that UTP therapy will also restore a portion of electrogenic $\text{HCO}_3^-$ secretion in CF epithelia expressing the $\text{Ca}^{2+}$-activated anion conductance pathway. This effect may be useful in normalizing transluminal pH across CF epithelia.

Murine CFTR knockout gallbladder provides a native epithelial model for the measurement of P2Y$_2$ receptor activation of the $\text{Ca}^{2+}$-mediated anion conductance. Previously, we have shown that murine gallbladder epithelial cells express P2Y$_2$ receptor mRNA and that the receptor complement is functionally localized to the luminal membrane (10). Furthermore, activation of the P2Y$_2$ receptor by UTP in murine gallbladder epithelium induces dose-dependent increases in inositol phosphate generation, intracellular $\text{Ca}^{2+}$ mobilization, and $I_{sc}$ (10). Characterization studies showed that the peak $I_{sc}$ response to UTP is independent of luminal Na$^+$, DIDS sensitive, reduced by removal of either Cl$^-$ or $\text{HCO}_3^-$ from the medium, and almost completely abolished by removal of both anions.

With the use of CFTR knockout mice in the present study, $I_{sc}$ measurements resulting from activation of the $\text{Ca}^{2+}$-activated anion conductance were uncomplicated by CFTR activity, a major conductive pathway for cAMP-mediated electrogenic anion secretion in the murine gallbladder (24, 29). In support of using the CF murine gallbladder for isolation of the $\text{Ca}^{2+}$-activated anion conductance, an epithelial $\text{Ca}^{2+}$-activated Cl$^-$ channel (mCLCA) has recently been cloned and was shown to be expressed at high levels in murine gallbladder (15, 17). Whether the mCLCA channel is solely responsible for the $\text{Ca}^{2+}$-activated anion channel activity demonstrated in earlier studies (26) of biliary epithelium has yet to be determined.

Two models have been proposed for electrogenic $\text{HCO}_3^-$ secretion across epithelial tissues. In one model, an inward current is generated by $\text{HCO}_3^-$ that permeates anion channels in the luminal membrane (1, 12). In a second model, the inward current is generated by Cl$^-$ permeation of luminal membrane anion channels. Cl$^-$ secretion in the latter model facilitates $\text{HCO}_3^-$ secretion by “recycling” Cl$^-$ entering via a luminal membrane Cl$^-$/$\text{HCO}_3^-$ exchanger (28). Several lines of evidence in the present study indicate that the plateau phase of the UTP-induced $I_{sc}$ response represents a $\text{HCO}_3^-$ secretory current carried by the $\text{Ca}^{2+}$-activated anion conductance (model 1). First, the plateau $I_{sc}$ response to UTP was not inhibited by bumetanide or complete Cl$^-$ substitution but required $\text{HCO}_3^-$ in the bathing medium. Second, the rates of UTP-induced $J_{s\rightarrow m}$ and $I_{sc}$ were similar in magnitude both under control conditions and during inhibition of Cl$^-$/$\text{HCO}_3^-$ exchange.
exchange by luminal Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} removal. Third, the UTP-stimulated increase in \(J_{\text{sc-\text{m}}}\) and \(J_{\text{sc}}\) during the plateau phase of the response could be completely inhibited by micromolar DIDS, a known blocker of the Ca\textsuperscript{2+}-activated anion conductance (4). Whether the recently cloned mCLCA channel mediates this HCO\textsubscript{3}\textsuperscript{−} conductance is not yet known. To the best of our knowledge, neither the mCLCA channel nor the Ca\textsuperscript{2+}-activated anion channels described in biliary epithelial cells (15, 26) have been evaluated for HCO\textsubscript{3}\textsuperscript{−} permeation.

Luminal Cl\textsuperscript{−} removal also revealed a significant amount of Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchange in the basal state of the CF murine gallbladder [for comparison, electroneutral HCO\textsubscript{3}\textsuperscript{−} secretion in the murine duodenum is less than one-third of this rate (9)]. If the residual \(J_{\text{sc-\text{m}}}\) in the absence of luminal Cl\textsuperscript{−} is taken as a measure of net paracellular flow of HCO\textsubscript{3}\textsuperscript{−} (\(-1\) μeq \cdot cm\textsuperscript{−2} \cdot h\textsuperscript{−1}), then the basal \(J_{\text{sc-\text{m}}}\) of the murine gallbladder would be \(~\sim 3\) and 4 μeq \cdot cm\textsuperscript{−2} \cdot h\textsuperscript{−1} in CF and wild-type mice, respectively. These values compare reasonably well with the basal \(J_{\text{sc-\text{m}}}\) measured across the gallbladder of rabbit and guinea pig (\(~\sim 3\) and \(~\sim 2\) μeq \cdot cm\textsuperscript{−2} \cdot h\textsuperscript{−1}, respectively) (30, 35). Note, however, that the direction of net HCO\textsubscript{3}\textsuperscript{−} movement (absorptive vs. secretory) across murine gallbladder in the basal state could not be ascertained because mucosal-to-serosal flux of HCO\textsubscript{3}\textsuperscript{−} was not measured. Nonetheless, Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchange apparently dominates the basal secretory flux of HCO\textsubscript{3}\textsuperscript{−} in the mouse gallbladder as it does in rabbit and guinea pig (30, 35). Although further studies will be necessary to determine the molecular identity of luminal Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchange, it was apparent from the studies shown in Fig. 5 that electroneutral HCO\textsubscript{3}\textsuperscript{−} secretion was not highly sensitive to luminal DIDS (\(~\sim 12\%\) decrease, not significant). This observation suggests that weakly DIDS-sensitive exchangers, such as the downregulated in adenoma protein (murine homologue) and the anion exchanger AE2 (2, 27), are candidate proteins for the luminal Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchange process in murine gallbladder.

In summary, it was shown that P2Y\textsubscript{2} receptor activation of a Ca\textsuperscript{2+}-mediated anion conductance results in electroneutral HCO\textsubscript{3}\textsuperscript{−} secretion across CF murine gallbladder epithelium. This finding suggests that nucleotide receptor therapy may restore in CF patients the loss of regulated HCO\textsubscript{3}\textsuperscript{−} secretion that is necessary for transmural pH modulation in certain organs. In comparison to CFTR-mediated HCO\textsubscript{3}\textsuperscript{−} secretion in murine gallbladder, \(~\sim 50\%\) of this activity can be induced by UTP treatment. However, the duration of the response is relatively short lived (30–60 min). Efforts to prolong activation of this pathway, perhaps by preventing P2Y\textsubscript{2} receptor desensitization (10), may be beneficial. As previously proposed for murine airway epithelia (8), the dominance of the Ca\textsuperscript{2+}-activated anion conductance in othermurine epithelial tissues may be responsible for the relative lack of pancreatic and biliary disease in CF mouse models. Thus increasing the activity and expression of components of the Ca\textsuperscript{2+}-activated anion conductance pathway in human epithelia is a potential treatment for cystic fibrosis.

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