Role of Down-Regulated in Adenoma Anion Exchanger in HCO_3^- Secretion Across Murine Duodenum

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Background & Aims: **The current model of duodenal HCO_3^- secretion proposes that basal secretion results from Cl^-/HCO_3^- exchange, whereas cyclic adenosine monophosphate (cAMP)-stimulated secretion depends on a cystic fibrosis transmembrane conductance regulator channel (Cftr)-mediated HCO_3^- conductance. However, discrepancies in applying the model suggest that Cl^-/HCO_3^- exchange also contributes to cAMP-stimulated secretion. Of 2 candidate Cl^-/HCO_3^- exchangers, studies of putative anion transporter-1 knockout (KO) mice find little contribution of putative anion transporter-1 to basal or cAMP-stimulated secretion. Therefore, the role of down-regulated in adenoma (Dra) in duodenal HCO_3^- secretion was investigated using DraKO mice.** ***Methods:*** **Duodenal HCO_3^- secretion was measured by pH stat in Ussing chambers. Apical membrane Cl^-/HCO_3^- exchange was measured by microfluorometry of intracellular pH in intact villous epithelium. Dra expression was assessed by immunofluorescence.** ***Results:*** **Basal HCO_3^- secretion was reduced ~55%–60% in the DraKO duodenum. cAMP-stimulated HCO_3^- secretion was reduced ~50%, but short-circuit current was unchanged, indicating normal Cftr activity. Microfluorimetry of villi demonstrated that Dra is the dominant Cl^-/HCO_3^- exchanger in the lower villous epithelium. Dra expression increased from villous tip to crypt. DraKO and wild-type villi also demonstrated regulation of apical Na^+/H^+ exchange by Cftr-dependent cell shrinkage during luminal Cl^- substitution.** ***Conclusions:*** **In murine duodenum, Dra Cl^-/HCO_3^- exchange is concentrated in the lower crypt-villus axis where it is subject to Cftr regulation. Dra activity contributes most basal HCO_3^- secretion and ~50% of cAMP-stimulated HCO_3^- secretion. Dra Cl^-/HCO_3^- exchange should be considered in efforts to normalize HCO_3^- secretion in duodenal disorders such as ulcer disease and cystic fibrosis.**

Duodenal mucosal HCO_3^- secretion, important in the formation of an alkaline mucus barrier against gastric acid, involves contributions by apical membrane Cl^-/HCO_3^- exchange activity and requires cystic fibrosis transmembrane conductance regulator (Cftr) activity for cyclic nucleotide–stimulated secretion.[1,2] The identity of the anion exchangers involved in duodenal HCO_3^- secretion has been a subject of investigation for several decades. Past immunolocalization studies have ruled out involvement of the SLC4 bicarbonate transporter SLC4A2 (anion exchanger isoform 2), which is expressed in intestinal epithelium but does not reside in the apical membrane compartment.[3] A recent addition to the family, SLC4a9 (anion exchanger 4 [Ae4]), has tentatively been localized to the apical membrane of murine duodenum but has very low levels of expression.[4,5] Rather, most evidence supports the involvement of the SLC26A family of multifunctional anion exchangers in duodenal Cl^-/HCO_3^- exchange. Of the 10 family members, 2 members have been localized to the apical membrane of intestinal epithelia in murine models. Down-regulated in adenoma (Dra; Slc26a3) exhibits high rates of Cl^-/HCO_3^- exchange, and loss-of-function mutations are responsible for the human genetic disease congenital Cl^--losing diarrhea.[6–9] Putative anion transporter-1 (Pat-1; Slc26a6) is a robust Cl^-/HCO_3^- exchanger but also exchanges sulfate, oxalate, and formate at lower rates.[10–12] Recent studies of murine duodenum indicate that Pat-1 is the dominant Cl^-/HCO_3^- exchanger in the upper villous epithelium.[5]

Although well recognized that duodenal HCO_3^- secretion involves the concerted activities of Cftr and anion exchanger(s), the relative contribution of each pathway to net HCO_3^- secretion under basal and cyclic nucleotide–stimulated conditions has been difficult to establish. Cftr has significant HCO_3^- permeability relative to Cl^- (~1:5, respectively[13]), increases Cl^-/HCO_3^- exchange activity by

Abbreviations used in this paper: Ae4, anion exchanger 4; cAMP, cyclic adenosine monophosphate; BCECF-AM, 2′,7′-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein acetoxymethyl ester; Cftr, cystic fibrosis transmembrane conductance regulator; dKO, double knockout; Dra, down-regulated in adenoma; EIPA, 5-(N-ethyl-n-isopropyl)-amiloride; G_t, transepithelial conductance; I_{sc}, short-circuit current; $J_{sm}^{HCO_3}$, serosal-to-mucosal bicarbonate flux; KBR, Krebs bicarbonate Ringer's solution; KO, knockout; mRNA, messenger RNA; Nhe3, Na^+/H^+ exchanger isoform 3; Pat-1, putative anion transporter-1; pH_i, intracellular pH; WT, wild-type.

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providing a Cl^- "leak" to recycle Cl^-,[14,15] and, when activated, alters cell volume and membrane potential.[16,17] Changes in cell volume regulate the activity of the apical membrane Na^+/H^+ exchanger Nhe3,[17] whereas changes in membrane potential may directly alter Cl^-/HCO_3^- exchange based on recently proposed electrogenic stoichiometries for PAT-1 (1 Cl^-:2 HCO_3^-) and DRA (2 Cl^-:1 HCO_3^-).[18] Particularly during stimulated secretion, these factors confound attempts to partition duodenal HCO_3^- secretion between a Cftr HCO_3^- conductance and Cl^-/HCO_3^- exchange.

In both human and murine duodena, most studies agree that basal rates of HCO_3^- secretion depend primarily on Cl^-/HCO_3^- exchange activity. Inhibition of Cl^-/HCO_3^- exchange by removal of luminal Cl^- abolishes basal HCO_3^- secretion and, although reduced, a finite rate of basal secretion is retained in the absence of Cftr activity.[2,19–21] In contrast, the role of Cl^-/HCO_3^- exchange during cyclic adenosine monophosphate (cAMP)-stimulated HCO_3^- secretion is less clear. Although a contribution of Cl^-/HCO_3^- exchange has been postulated,[2] studies in which apical membrane Cl^-/HCO_3^- exchange is inhibited by luminal Cl^- removal have concluded that most cAMP-stimulated secretion involves a Cftr-mediated HCO_3^- conductance.[2,21] This is further complicated by recent studies showing that the HCO_3^- permeability of recombinant CFTR may be increased in the absence of extracellular Cl^-.[22] Further, two studies of murine duodenal HCO_3^- secretion have yielded indirect evidence of a significant contribution of Cl^-/HCO_3^- exchange during cAMP stimulation when physiologic concentrations of Cl^- are present in the luminal bath. In the first study,[2] inhibition of carbonic anhydrase activity reduced the rate of HCO_3^- secretion by 50% without altering the transepithelial short-circuit current (I_{sc}), an index of Cftr activity. In a second study,[23] measurements of serosal-to-mucosal HCO_3^- and Cl^- flux found that only 50% of cAMP-stimulated HCO_3^- secretion was associated with the I_{sc} during loss of $Na^+/K^+/2$ Cl^- cotransporter Nkcc1 activity. In efforts to identify the relevant Cl^-/HCO_3^- exchangers contributing to HCO_3^- secretion, recent investigations of Pat-1 knockout (KO) duodenum show that Pat-1 only provides ~20% of basal HCO_3^- secretion and no contribution to cAMP-stimulated secretion.[24] Thus, it is imperative that the role of the other major Cl^-/HCO_3^- exchanger, Dra, be investigated for its contribution to duodenal HCO_3^- secretion.

Identifying the role of apical membrane Cl^-/HCO_3^- exchangers is fundamental to creating an accurate model of duodenal HCO_3^- secretion. The model depends upon studies of native intestinal mucosa, but specific inhibitors or stimulants that effectively discriminate between the exchanger isoforms are not available or exert additional effects on other anion transport proteins. Therefore, in the present study, we performed ex vivo duodenal studies using mice with gene-targeted deletion of Dra to assess its contribution to transepithelial HCO_3^- secretion.

BASIC–ALIMENTARY TRACT

Materials and Methods

Animals

Mice with the gene-targeted disruptions of murine *Slc26a3* (*Dra*),[25] *Slc26a6* (*Pat-1*, a gift from M. Soleimani, University of Cincinnati Medical Center, Cincinnati, OH),[24] *Abcc7* (*Cftr*),[26] or *Slc4a9* (*Ae4*, a gift from G.E. Shull, University of Cincinnati)[5] on a mixed genetic background were used. All comparisons of homozygous KO mice were made with gender- and age-matched (+/+) siblings (wild-type [WT]). Mutant mice were identified using polymerase chain reaction–based analysis of tail-snip DNA.[27] Mice were maintained on standard laboratory chow (Formulab 5008 Rodent Chow; Purina, St. Louis, MO) and tap water until the day before an experiment. The drinking water of DraKO (and WT littermate) mice routinely contained 50% Pedialyte (Abbott, Columbus, OH) to prevent dehydration secondary to diarrhea.[25] Mice (2–4 months) were fasted overnight before experimentation but were provided water *ad libitum*. The mice were singly housed in a temperature (22°C–26°C) and light (12:12-hour light-dark)-controlled room in the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited animal facility at the Dalton Cardiovascular Research Center. All experiments involving animals were approved by the University of Missouri Animal Care and Use Committee.

pH Stat

The method for pH stat measurement of transepithelial HCO_3^- secretion in murine duodenum has been previously described.[23] Excised proximal duodenum was stripped of external muscle layers and mounted on Ussing chambers (0.25 cm^2 aperture). Neural activity and prostaglandin generation were blocked with tetrodotoxin (0.1 μmol/L, serosal) and indomethacin (1 μmol/L, bilateral). Spontaneous potential difference was voltage-clamped to 0 mV, allowing measurement of the transepithelial I_{sc} (μeq/cm^2 tissue surface area · h) and conductance (G_t, mS/cm^2). All experiments were carried out under short-circuited conditions with the serosal bath serving as ground. The mucosal surface was bathed with unbuffered NaCl solution and vigorously gassed with 100% O_2. The serosal-to-mucosal bicarbonate flux ($J_{sm}^{HCO_3}$) was measured by clamping luminal bath pH at 7.4 using 5 mmol/L HCl administered by an automatic titrator (Radiometer Analytical, Lyon, France). In some experiments, Cl^- content of the luminal solution was replaced equimolar using $gluconate^-$ and $isethionate^-$. The serosal bath contained Krebs bicarbonate Ringer's solution (KBR) containing 10 mmol/L glucose (pH 7.4; gassed with 95% O_2:5% CO_2; 37°C). Subsequent experiments consisted of two 30-minute flux periods: an untreated period (Basal), followed by a treatment period (cAMP) beginning 15 minutes after bilateral 10 μmol/L forskolin addition.

Intracellular pH Measurement

The method used for imaging villous epithelial cells in intact murine duodenal mucosa has been previously described.[5,15] Briefly, muscle-stripped duodenum was