Down-regulated in Adenoma Cl/HCO_3 Exchanger Couples With Na/H Exchanger 3 for NaCl Absorption in Murine Small Intestine

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Background & Aims: **Electroneutral NaCl absorption across small intestine contributes importantly to systemic fluid balance. Disturbances in this process occur in both obstructive and diarrheal diseases, eg, cystic fibrosis, secretory diarrhea. NaCl absorption involves coupling of Cl^-/HCO_3^- exchanger(s) primarily with Na^+/H^+ exchanger 3 (Nhe3) at the apical membrane of intestinal epithelia. Identity of the coupling Cl^-/HCO_3^- exchanger(s) was investigated using mice with gene-targeted knockout (KO) of Cl^-/HCO_3^- exchangers: Slc26a3, down-regulated in adenoma (Dra) or Slc26a6, putative anion transporter-1 (Pat-1).** ***Methods:*** **Intracellular pH (pH_i) of intact jejunal villous epithelium was measured by ratiometric microfluoroscopy. Ussing chambers were used to measure transepithelial $^{22}Na^{36}Cl$ flux across murine jejunum, a site of electroneutral NaCl absorption. Expression was estimated using immunofluorescence and quantitative polymerase chain reaction.** ***Results:*** **Basal pH_i of DraKO epithelium, but not Pat-1KO epithelium, was alkaline, whereas pH_i in the Nhe3KO was acidic relative to wild-type. Altered pH_i was associated with robust Na^+/H^+ and Cl^-/HCO_3^- exchange activity in the DraKO and Nhe3KO villous epithelium, respectively. Contrary to genetic ablation, pharmacologic inhibition of Nhe3 in wild-type did not alter pH_i but coordinately inhibited Dra. Flux studies revealed that Cl^- absorption was essentially abolished (>80%) in the DraKO and little changed (<20%) in the Pat-1KO jejunum. Net Na^+ absorption was unaffected. Immunofluorescence demonstrated modest Dra expression in the jejunum relative to large intestine. Functional and expression studies did not indicate compensatory changes in relevant transporters.** ***Conclusions:*** **These studies provide functional evidence that Dra is the major Cl^-/HCO_3^- exchanger coupled with Nhe3 for electroneutral NaCl absorption across mammalian small intestine.**

The small intestinal process of transepithelial NaCl absorption is known to involve the coupled activity of Na^+/H^+ and Cl^-/HCO_3^- exchangers.[1,2] Electroneutral NaCl absorption (so-called because absorption is not associated with transepithelial electrical currents) provides a mechanism for copious NaCl and water absorption across the intestine, especially under physiologic conditions such as the stress response. Earlier studies to identify the Na^+/H^+ exchanger (NHE) involved in coupled NaCl absorption established that SLC9A3, ie, NHE3, is the principal Na^+/H^+ exchanger isoform responsible for transepithelial Na^+ absorption at the apical membrane of intestinal epithelia. The evidence for predominance of NHE3 in transepithelial Na^+ absorption came, by necessity, from studies of native intestinal mucosa using isoform-specific pharmacologic inhibitors and measurements of transepithelial isotopic flux across Nhe2 and Nhe3 knockout (KO) mouse intestine.[3–5] Other NHE isoforms, NHE2 (SLC9A2) and NHE8 (SLC9A8), have also been reported to contribute but have less dominant roles under basal conditions in the small intestine.[3,6,7]

Recent studies indicate that coupled Cl^-/HCO_3^- exchanger(s) for NaCl absorption are likely members of the SLC26A family of multifunctional anion exchangers. Of the 10 family members, 2 members have been localized to the apical membrane of mammalian intestinal epithelia. SLC26A3, also known as down-regulated in adenoma (DRA), normally exhibits high rates of Cl^-/HCO_3^- exchange, and loss-of-function mutations of SLC26A3 are responsible for the human genetic disease congenital Cl^- losing diarrhea (CLD).[8–10] SLC26A6, also known as PAT-1, is a robust Cl^-/HCO_3^- exchanger but also exchanges sulfate, oxalate, and formate at lower rates.[11–13] Some, but not all,[14] studies of recombinant DRA and PAT-1 provide evidence that these transporters may have

Abbreviations used in this paper: Cftr, cystic fibrosis transmembrane conductance regulator; CLD, congenital chloride-losing diarrhea; Dra, down-regulated in adenoma; EIPA, 5-(N-ethyl-n-isopropyl)-amiloride; G_t, transepithelial conductance; I_{sc}, short-circuit current; J, ion flux; KO, knockout; M-S, mucosal-to-serosal; NFA, niflumic acid; Nhe3, Na^+/H^+ exchanger isoform 3; S-M, serosal-to-mucosal; Pat-1, putative anion transporter-1.

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stoichiometries consistent with electrogenicity (PAT-1 $1Cl^-$:2 HCO_3^-; DRA $2Cl^-$:1HCO_3^-).[12,15] Based on the opposing stoichiometries, it has been further proposed that electroneutrality of coupled NaCl absorption is preserved by paired cellular operation of the 2 anion exchangers.[15]

Although classical descriptions of coupled NaCl absorption have come from studies of the small intestine, notably rabbit ileum,[16] studies of colonic epithelium have provided indirect evidence that Dra is the major Cl^-/HCO_3^- exchange involved in coupled NaCl absorption. Investigations of CLD patients led to the elucidation of DRA as a major anion exchanger in the large intestine,[8] which has been confirmed using brush-border membrane vesicles from colonic epithelium of DraKO mice.[17] Evidence that Dra couples with Nhe3 for NaCl absorption was provided in studies showing that Dra expression is up-regulated in the Nhe3KO colon[18] and that Nhe3 expression is up-regulated in the DraKO mouse colon (although the latter finding may be complicated by the effects of hyperaldosteronism secondary to dehydration).[17] In contrast, less is known about the identity of the coupled Cl^-/HCO_3^- exchanger in small intestine. The available findings indicate several differences from colonic NaCl absorption. Up-regulation of Dra expression was not detected in the Nhe3KO small intestine, and overt changes in crypt morphology found in the DraKO colon were not evident in the small bowel.[17,18] In addition, the expression of Dra and Nhe3 is less in the small intestine as compared with the colon, and Pat-1, which is weakly expressed in the large intestine, presents an additional possibility for coupling with Nhe3.[18,19]

Murine jejunum provides a useful model of coupled NaCl absorption in the small intestine by showing high rates of electroneutral NaCl absorption similar to that described in studies of human ileum, rat jejunum, and rabbit ileum.[1,16,20–22] Identification of the apical membrane Cl^-/HCO_3^- exchanger(s) involved in coupled NaCl absorption across the small intestine requires that studies be performed using native intestine. Murine jejunum has the advantages of providing sufficient intestinal preparations for bidirectional isotopic NaCl flux measurements and the opportunity to perform studies on mice with gene-targeted KO of the anion exchangers, which is important in that specific inhibitors or stimulants that discriminate between Slc26a isoforms are not available. Therefore, in the present study, ex vivo intestinal studies were performed using mice with gene-targeted deletions of Pat-1 and Dra to assess their relative contribution to coupled NaCl absorption across the small intestine.

Materials and Methods

Animals

The experiments in this study were performed using mice with gene-targeted disruptions of the murine homologs of *Slc26a3* (*Dra*),[17] *Slc26a6* (*Pat-1*),[23] or *Slc9a3(Nhe3)*[5] on a mixed genetic background. All comparisons of homozygous KO (−/−) mice were made with sex- and age-matched (+/+) siblings (wild-type; WT). The mutant mice were identified by using a polymerase chain reaction (PCR)-based analysis of tail snip DNA, as previously described.[20] All mice were maintained ad libitum on standard laboratory chow (Formulab 5008 Rodent Chow; Purina Mills, St Louis, MO) and tap water. The drinking water of the DraKO, Nhe3KO and WT littermate mice routinely contained 50% strength Pedialyte (Abbott Nutrition, Columbus, OH) to prevent dehydration in the KO mice secondary to diarrhea.[17] Analysis of blood from Pedialyte-treated DraKO mice did not reveal a difference in hematocrit from WT mice. There was a small, but nonsignificant, increase in blood pH (WT, 7.17 vs DraKO, 7.26) coincident with moderately increased HCO_3^- concentration and pCO_2, indicating a degree of metabolic alkalosis with respiratory compensation (Supplementary Table 1; see Supplementary material online at www.gastrojournal.org). Mice (age, 2–4 months) were fasted overnight prior to experimentation but were provided with drinking water ad libitum. The mice were housed in the AAALAC-accredited Dalton Cardiovascular Research Center animal facility. All experiments involving animals were approved by the University of Missouri Animal Care and Use Committee.

Transepithelial $^{22}Na^{36}Cl$ Flux Analysis

The method for transepithelial $^{22}Na^{36}Cl$ flux across murine jejunum has been previously described.[24] Briefly, midjejunal sections were stripped of external muscle layers before mounting on Ussing chambers (0.25-cm^2 surface area). Transepithelial short-circuit current (I_{sc}; $\mu Eq/cm^2 \cdot h$) and transepithelial conductance (G_t; mS/cm^2) were measured using an automatic voltage clamp (VCC-600; Physiologic Instruments, San Diego, CA). Mucosal and serosal sides of each section were independently bathed with Krebs bicarbonate Ringers solution (KBR) gassed with 95% O_2:5% CO_2 (pH 7.4; 37°C). In some experiments to evaluate the effect of low luminal HCO_3^- concentration on Cl^- absorption, HCO_3^- in the luminal bath was substituted with the buffer TES (25 mmol/L) and gassed with 100% O_2 (pH 7.4). All ex vivo preparations were treated with indomethacin (1 μmol/L, bilateral) and tetrodotoxin (0.1 μmol/L, serosal) to minimize the effects of endogenous prostaglandins and neural tone, respectively.[25,26] Approximately 5 μCi of ^{22}Na and $^{36}C1$ were added to the "source" bathing medium, and, following a 30-minute equilibration period, triplicate aliquots (200 μL) were taken from the "sink" side at the beginning and end of the 30-minute flux period. To determine the effect of cAMP on NaCl absorption, intracellular cAMP was stimulated by the bilateral addition of 10 μmol/L forskolin and 100 μmol/L isobutylmethyl xanthine for 30 minutes prior to