Mutations in human DRA cause congenital chloride diarrhea, thereby raising the possibility that it functions as a Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchanger. To test this hypothesis we cloned a cDNA encoding mouse DRA (mDRA) and analyzed its activity in cultured mammalian cells. When expressed in HEK 293 cells, mDRA conferred Na\(^{+}\)-independent, electroneutral Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchange activity. Removal of extracellular Cl\(^{-}\) from medium containing HCO\(_3\)\(^{-}\) caused a rapid intracellular alkalization, whereas the intracellular pH increase following Cl\(^{-}\) removal from HCO\(_3\)\(^{-}\)-free medium was reduced greater than 7-fold. The intracellular alkalization in Cl\(^{-}\)-free, HCO\(_3\)\(^{-}\)-containing medium was unaffected by removal of extracellular Na\(^{+}\) or by depolarization of the membrane by addition of 75 mm K\(^{+}\) to the medium. Like human DRA mRNA, mDRA transcripts were expressed at high levels in cecum and colon and at lower levels in small intestine. The expression of mDRA mRNA was modestly up-regulated in the colon of mice lacking the NHE3 Na\(^{+}/\)H\(^{+}\) exchanger. These results show that DRA is a Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchanger and suggest that it normally acts in concert with NHE3 to absorb NaCl and that in NHE3-deficient mice its activity is coupled with those of the sharply up-regulated colonic H\(^{+}\),K\(^{+}\)-ATPase and epithelial Na\(^{+}\) channel to mediate electrolyte and fluid absorption.

**Mouse Down-regulated in Adenoma (DRA) Is an Intestinal Cl\(^{-}/\)HCO\(_3\)\(^{-}\) Exchanger and Is Up-regulated in Colon of Mice Lacking the NHE3 Na\(^{+}/\)H\(^{+}\) Exchanger**

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Human DRA,\(^{1}\) cloned from a colon cDNA subtraction library, is expressed in the normal colon but not in most adenocarcinomas, thus the term Down-Regulated in Adenoma (1). Although it was originally proposed as a candidate tumor suppressor protein, there is only a slight increase in the incidence of intestinal cancer among individuals carrying mutations in the gene (2). Recent studies have shown that DRA is related to the sulfate transporters, DTDST (3) and Sat-1 (4) and that it produces a DIDS-sensitive, Na\(^{+}\)-independent sulfate transport activity when expressed in *Xenopus* oocytes (5) or Sf9 insect cells (6). It is unclear, however, whether sulfate transport is the major physiological function of DRA. Studies of humans with congenital chloride diarrhea (CLD), for which there is strong evidence of a defect in Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchange in the ileum and colon (7–9), revealed that the disease was caused by null mutations in the DRA gene (10, 11). This suggests that DRA might be a major transporter involved in Cl\(^{-}\) absorption in the colon. Consistent with this hypothesis, Moseley *et al.* (12) have shown that DRA mediates Cl\(^{-}\) uptake when expressed in *Xenopus* oocytes. These experiments, however, did not allow a determination of whether the Cl\(^{-}\) transport mechanism was Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchange.

The colon is a major site for NaCl absorption in the gastrointestinal tract, and much of this activity appears to be mediated by coupled Na\(^{+}/\)H\(^{+}\) and Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchange (13–15). Two Na\(^{+}/\)H\(^{+}\) exchangers, NHE2 and NHE3, are expressed on apical membranes of epithelial cells in the small intestine and colon (16, 17). Diarrhea occurs in mice lacking NHE3 (18) but not in mice lacking NHE2 (19). These observations indicate that NHE3 is the major Na\(^{+}/\)H\(^{+}\) exchanger contributing to NaCl absorption via coupled Na\(^{+}/\)H\(^{+}\) and Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchange; however, the molecular identity of the anion exchanger involved in NaCl absorption is uncertain. The AE2 Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchanger is expressed throughout the gastrointestinal tract (20) and was identified by Western blot analysis in brush border membranes from ileum (21). Thus, it is conceivable that this isoform might be responsible, at least in part, for the apical Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchange activity in small intestine. However, the membrane location of AE2 in colonic epithelial cells has not been determined, and immunolocalization studies have shown that it is expressed on basolateral membranes in epithelial cells of the stomach (22), renal nephron (23), and salivary glands (24). Furthermore, the severe deficiency in Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchange in CLD patients suggests that AE2 is not the major apical Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchanger in the ileum and colon.

DRA is highly expressed in colon and cecum and to a lesser extent in the small intestine (5, 10) and is localized to the apical membranes of colonic surface cells and the upper regions of the crypts (25). It is therefore reasonable to propose that it might be responsible for the Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchange activity that has been identified in normal colon (15, 26) and shown to be absent in CLD patients (7–9). To test the hypothesis that DRA mediates Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchange, we have cloned and functionally expressed mouse DRA. Our data demonstrate that mDRA, which is unrelated to members of the AE Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchanger
family, mediates Na\(^+\)-independent, electroneutral Cl\(^-\)/HCO\(_3\)\(^-\) exchange when expressed in mammalian cells. When considered in the light of previous studies of DRA and CLD patients, the results of this study suggest that DRA is the major apical Cl\(^-\)/HCO\(_3\)\(^-\) exchanger of the intestinal epithelium.

**EXPERIMENTAL PROCEDURES**

Cloning of the Mouse DRA cDNA—A fragment of the mDRA cDNA was amplified from mouse colon cDNA using a primer based on sequences for codons 474–481 of hDRA (1) and the sequence complementary to codons 84–93 that was obtained from a 310-base pair mDRA expression sequence tag (accession number AA217241). Mouse sequences from this fragment were used in a RACE procedure to clone the complete mDRA cDNA. Mouse colon mRNA was reverse-transcribed using oligo(dT) to generate cDNA corresponding to the 3' end of the mDRA mRNA, and an mDRA-specific primer corresponding to codons 102–108 to generate cDNA corresponding to the 5' end of the mRNA. Second strand cDNA synthesis was then performed, and Marathon cDNA adapters were ligated to each cDNA sample according to the manufacturer's recommendations (Marathon cDNA amplification kit, CLONTECH, Palo Alto, CA). 5' and 3' RACE reactions were performed using the adapter-ligated colon cDNA samples as templates. To amplify sequences corresponding to the 5' region of the mDRA mRNA we used the primer CATTCTCAGCTGAACTTCTATAGGGC and amplified with the Marathon kit and the mDRA-specific primer complementary to codons 102–109. To amplify sequences corresponding to the 3' end of the mDRA mRNA we used the AP1 primer and an mDRA-specific primer corresponding to codons 247–254. Secondary PCR reactions were then performed using a nested AP2 primer (5'-ACTTCAGCATTAGGCTGCTGGCCG-3') and either an mDRA primer complementary to codons 95–101, to amplify the 5' region, or an mDRA corresponding to codons 443–449, to amplify the 3' region. RACE amplification products were directly sequenced on an automatic sequencer (ABI Prism 377, Foster City, CA).

Nucleotide sequences from the 5'- and 3'-untranslated regions were then used to design primers (underlined in Fig. 1) to amplify and clone the entire coding region of mDRA by reverse transcription-PCR using Advantage Klentaq polymerase mix (CLONTECH). The sequence 5'-CCGCTCGAGCG-3' was added at the 5' end of the antisense primer. The PCR product containing the mDRA open reading frame was digested with XhoI and subcloned into the eukaryotic expression vector, pcIneo (Promega, Madison, WI), previously digested with EcoRI and SalI. After ligation the 5' ends of the mDRA insert into the SalI site of pcIneo (SalI overhangs are compatible with those generated with XhoI), the free ends of the construct were filled in using the Klenow fragment of DNA polymerase I to permit ligation of the 5' end of the insert with the blunt-ended EcoRI site of the vector. Sequence analysis was performed using MacVector software (International Biotechnologies Inc., New Haven, CT).

**Northern Hybridization Analysis**—Total RNA was isolated from intestinal segments (duodenum, jejenum, ileum, cecum, proximal colon, and distal colon) of adult Nhe3\(^+/-\) mice using Tri Reagent\(^{TM}\) as described by the supplier (Molecular Research Center, Inc., Cincinnati, OH). Each RNA sample was isolated from pooled tissues from 3 mice. A blot was prepared using 10 \(\mu\)g of RNA per lane and examined by UV shadowing to ensure equal loading of RNA sample. The blot was analyzed with a 396-base pair mDRA probe spanning codons 559–691 using hybridization and washing conditions described previously (19). After autoradiography and quantitation of hybridization intensities by PhosphorImager analysis (Molecular Dynamics, Wayzata, MN), the blot was stripped, hybridized with a 1.6-kb rat AE2 probe spanning codons 456 to 1002, and analyzed by autoradiography and PhosphorImager analysis.

**Transfection and Stable Expression of mDRA**—The orientation and coding region of the pcIneo/mDRA construct were verified by sequencing of three different isolates. Cl\(^-\)/HCO\(_3\)\(^-\) exchanger-deficient HEK 293 cells were maintained in Dulbecco's modified Eagle's medium (Life Technologies, Inc.) supplemented with 10% fetal bovine serum, 2 mM l-glutamine, and penicillin (50 units/ml) streptomycin (100 \(\mu\)g/ml). 293 cells were transfected with 20 \(\mu\)g of pcIneo/mDRA by the CaPO\(_4\) DNA co-precipitation method (27). Cells were selected for stable expression with G418 (1,000 units/ml, Life Technologies, Inc.). Intracellular Cl\(^-\) Measurements—Intracellular Cl\(^-\) was monitored using the pH-sensitive dye BCECF (Molecular Probes, Eugene, OR) as described previously (28). Briefly, transfected cells plated on glass coverslips were attached to the base of a superfusion chamber mounted on a Nikon diaphot microscope interfaced with an AR-CM microfluorimeter (SPEX Industries, NJ). Coverslips were superfused with a HCO\(_3\)\(^-\)-containing, Cl\(^-\)-free salt solution to produce an alkaline load in the presence of functional Cl\(^-\)/HCO\(_3\)\(^-\) exchange activity (29). The physiological salt solution contained sodium HCO\(_3\) (25), NaCl (150), KO\(_2\) (4), glucose (10), Hepes (20), CaCl\(_2\) (1.2), MgCl\(_2\) (0.8), pH 7.4. Chloride salts were replaced with gluconate salts in the Cl\(^-\)-free solutions and NaHCO\(_3\) was replaced with sodium gluconate in the HCO\(_3\)\(^-\)-free solutions. For the Na\(^-\)free solution, Na\(^+\) was replaced with N-methyl-D-glucamine, and for the high K\(^+\)-containing solution, 70 mM NaCl was replaced by KCl. Rates of change were determined from the initial linear portion of the fluorescence trans following the removal of extra-cellular Cl\(^-\) and expressed as mean \(\pm\) S.E. The intracellular pH signal was calibrated by the high potassium-nigericin technique (30).

**RESULTS**

Isolation and Characterization of the Mouse DRA cDNA—To clone the mDRA cDNA we used a combination of RACE and reverse transcription-PCR protocols. The full-length coding region of the 757-amino acid mDRA protein was inserted into the pcIneo mammalian expression vector, and the sequences of three independent isolates of the pcIneo/mDRA construct were determined. The nucleotide sequences of isolates 1 and 2 were identical, but the sequence of clone 3 contained four nucleotide substitutions. Two of the substitutions were in the third position of the codon (T to C in codons 471 and 690) and did not alter the amino acid sequence, whereas the remaining substitutions (G to A) converted Ala-93 to Thr and Ala-483 to Thr. The corresponding hDRA cDNA sequences encode Ala residues, as in mDRA cDNA isolates 1 and 2. The Ala to Thr sequence variations in clone 3 could be the result of Klentaq polymerase replication errors or, alternatively, may be naturally occurring polymorphisms. If they are polymorphisms then they appear to be functionally neutral since expression of both clones 1 and 3 resulted in Cl\(^-\)/HCO\(_3\)\(^-\) exchange activity (see below).

The nucleotide and deduced amino acid sequences of the mDRA cDNA are shown in Fig. 1. The translation initiation site matches that of hDRA, and with an Ala residue in the -3 position it is in a good context for initiation of translation (31). Two in-frame stop codons were present before the initiation methionine, as noted for the hDRA 5'-untranslated region (1), and there were no upstream ATG codons. The 5' and 3'-untranslated sequences of mDRA (spanning ~350 nucleotides) were 76 and 84% identical, respectively, to the corresponding untranslated sequences of hDRA, and few gaps were required to align the sequences (data not shown).

The 757-amino acid sequence of mDRA is 81% identical to hDRA (Fig. 2) and, if conservative substitutions are considered, the two proteins exhibit 95% amino acid similarity. The first 138 amino acids and the last 586 amino acids of the two proteins can be aligned without gaps, however, there is considerable sequence divergence in the putative second extracellular loop between transmembrane domains 3 and 4 (corresponding to human codons 139–178) where 7 amino acids are absent in mDRA. DRA is expressed as a glycoprotein in human colon (25). It is not known if mDRA is a glycoprotein; however, two of the four potential N-glycosylation sites that are present in the second extracellular loop of hDRA (Asn-153, -161, -164, and -165) are also present in mDRA (Asn-149 and -161, corresponding to hDRA Asn-153 and -155).

In view of the data described below, showing that DRA is an electroneutral Cl\(^-\)/HCO\(_3\)\(^-\) exchanger, we performed amino acid alignments to determine whether it was related to the AE Cl\(^-\)/HCO\(_3\)\(^-\) exchanger family. These comparisons (data not shown) revealed no significant similarity to AE1, AE2, or AE3, indicating that DRA does not share a common ancestral protein with the AE isoforms.

**Functional Expression of mDRA**—To determine whether mDRA can function as a Cl\(^-\)/HCO\(_3\)\(^-\) exchanger, the open-
ing frame of mDRA was subcloned into the expression vector pCIneo and two distinct isolates (clones 1 and 3) were functionally expressed in HEK 293 cells. 

Cl2/HCO32 exchange can be examined directly by reversing the Cl2 gradient in HCO32-containing medium and monitoring intracellular pH (29). In the presence of a Cl2/HCO32 exchanger, an intracellular alkalinization occurs due to the accumulation of HCO32. Alkalinization was not observed in mock-transfected control HEK 293 cells (Fig. 3, upper panel). In contrast, a rapid intracellular alkalinization was activated in cells stably transfected with mDRA clone 1 following the removal of extracellular Cl2, and this alkalinization was reversible (Fig. 3, upper panel). Clone 3 contained nucleotide substitutions that resulted in the translation of two different amino acids (Ala-93 to Thr and Ala-483 to Thr). These conservative substitutions were functionally neutral because expression of this latter clone resulted in Cl2/HCO32 exchange activity (Fig. 3, lower panel).

mDRA exhibited only a low sensitivity to the anion transport inhibitor DIDS, with 24±63% inhibition at a concentration of 1 mM (Fig. 4, upper panel). We repeated the above experiments in the absence of extracellular HCO32. This maneuver ordinarily eliminates the anion exchange-mediated intracellular alkalinization that occurs when extracellular Cl− is removed (29, 34). Removal of HCO32 dramatically decreased the alkalinization rate in cells expressing mDRA (Fig. 4, lower panel); however, it should be noted that this manipulation did not eliminate the alkalinization. The intracellular alkalinization that was resistant to removal of HCO32 (13±4% of the initial rate of alkalinization observed in HCO32-containing medium) may represent the transport of hydroxyl ions and/or gluconate, consistent with the possibility that the extracellular anion site is relatively nonspecific. It should also be noted that re-addition of extracellular Cl− in the absence of HCO32 did not lead to the rapid pH recovery that occurred in medium containing HCO32 (Fig. 4, lower panel).

Anion exchange in mammalian cells is typically electroneutral and not dependent on extracellular Na+ (29, 32). One exception, however, is the Na+/H+ exchanger, Na+-dependent Cl2/HCO32 exchange that has been described in several cell types (33–35). The upper panel of Fig. 5 shows that mDRA-mediated Cl2/HCO32 exchange is Na+ independent. Moreover, anion exchange in mDRA-expressing cells was insensitive to changes in the membrane potential. The lower panel of Fig. 5 shows that depolarization of the plasma membrane by high extracellular K+ failed to alter the rate or magnitude of the alkalinization induced by Cl− removal.

**Fig. 1.** Nucleotide and deduced amino acid sequence of mDRA. Nucleotides and amino acids are numbered on the right. The underlined untranslated sequences (nucleotides −77 to −53 and 2283 to 2307) correspond to the sense and antisense primers, respectively, used to amplify the mDRA coding sequence used to generate the expression construct pCIneo/mDRA.
serve as a compensatory mechanism, Northern blot analysis of intestinal segments from Nhe3\(^{-/-}\) and Nhe3\(^{-/-}\) mice were performed. The 3.2-kb mDRA mRNA was expressed at lower levels in small intestine than in cecum and colon (Fig. 6, top and middle panels). DRA mRNA levels were similar in each segment of the small intestine and were slightly reduced in Nhe3\(^{-/-}\) mice (~25%, 7%, and 25% lower, respectively, in duodenum, jejunum, and ileum). mDRA was expressed at much higher levels in cecum, proximal colon, and distal colon. The level of mDRA mRNA in cecum was only slightly elevated in Nhe3\(^{-/-}\) mice (~20% greater than in Nhe3\(^{-/-}\) mice), but was ~75% greater in proximal colon and ~50% greater in distal colon.

The same blot was analyzed using a probe for the AE2 Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchanger. AE2 expression patterns were similar to those of mDRA, with much lower mRNA levels in small intestine than in cecum and colon (Fig. 6, bottom panel). Longer exposure times, however, were required to detect AE2 mRNA, even though the AE2 probe was considerably longer than the DRA probe. This suggests that the levels of AE2 mRNA in all intestinal segments are lower than those of DRA. Relative to the levels observed in Nhe3\(^{-/-}\) mice, AE2 mRNA levels were slightly reduced in small intestinal segments of Nhe3\(^{-/-}\) mice (decreased by ~10% in duodenum, 5% in jejunum, and 10% in ileum). AE2 mRNA levels in cecum and proximal colon were increased by ~50 and 40%, respectively, in NHE3-deficient mice and were unchanged in distal colon.

**DISCUSSION**

Congenital chloride diarrhea is characterized by an acidic, watery stool containing high concentrations of Cl\(^{-}\) and low concentrations of HCO\(_3\)\(^{-}\) (36). In the absence of appropriate therapy, affected patients exhibit hypochloremia, hypokalemia, and metabolic alkalosis (36), resulting from a deficit in intestinal Cl\(^{-}\) absorption (7, 8, 37). Human genetics studies showed that mutations in the DRA gene are responsible for CLD (10, 11), and functional expression studies demonstrated that DRA mediates uptake of sulfate, oxalate, and Cl\(^{-}\) (5, 12) and that the related Sat-1 protein functions as a SO\(_4\)\(^{2-}/\)HCO\(_3\)\(^{-}\) exchanger (3, 38). However, the ion transport activity of the protein was not clearly established. Intestinal perfusion studies suggested that apical Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchange was absent in the ileum (7) and colon (8, 9) of CLD patients but did not exclude the possibility that DRA contributed to this process as part of a coupled system. Thus, our major objective was to test the hypothesis that DRA functions as a Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchanger.

To examine this issue we analyzed the activity of the transporter in stably transfected HEK 293 cells. Reversal of the Cl\(^{-}\) gradient by removal of extracellular Cl\(^{-}\) in the presence of extracellular HCO\(_3\)\(^{-}\) led to a rapid alkalization of cells expressing DRA, and intracellular pH recovered quickly when Cl\(^{-}\) was restored to the extracellular medium. In contrast, when removal of Cl\(^{-}\) was performed using HCO\(_3\)\(^{-}\)-free medium, the rate of increase in intracellular pH was sharply reduced. These data show that HCO\(_3\)\(^{-}\) is efficiently transported into the cell in response to an outwardly directed Cl\(^{-}\) gradient and that the process is reversed when extracellular Cl\(^{-}\) is restored. The observed alkalization during Cl\(^{-}\) removal in the presence of HCO\(_3\)\(^{-}\) was not affected by extracellular Na\(^{+}\) or by depolarization of the membrane. On the basis of these data, we conclude that mDRA can function as an electroneutral, Na\(^{+}\)-independent Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchanger.

The low rate of alkalization that occurred during Cl\(^{-}\) removal in the absence of extracellular HCO\(_3\)\(^{-}\) is intriguing and raises the possibility that other anions present in the extracellular medium, such as OH\(^{-}\) and/or gluconate, might be transported by DRA. There is precedent for this possibility, as there is evidence that AE2 transports gluconate at a low rate (39). These findings and the studies showing that DRA mediates uptake of sulfate, oxalate, and chloride when expressed in Xenopus oocytes (5, 12) suggest that the anion specificity of its
The physiological data showing that apical Cl\textsuperscript{-}/HCO\textsubscript{3}\textsuperscript{-} exchange is absent in ileum and colon of CLD patients (7–9) are compelling. Although it has been suggested that Cl\textsuperscript{-}/HCO\textsubscript{3}\textsuperscript{-} exchange might be reversed in CLD patients (41), Bieberdorf et al. (7) demonstrated that the decrease in HCO\textsubscript{3}\textsuperscript{-} observed over time in the ileum of CLD patients perfused with HCO\textsubscript{3}\textsuperscript{-}-containing fluids was not due to direct HCO\textsubscript{3}\textsuperscript{-} absorption, but rather to H\textsuperscript{+} secretion and subsequent neutralization of HCO\textsubscript{3}\textsuperscript{-}, as indicated by increased pCO\textsubscript{2} in the luminal perfusate as HCO\textsubscript{3}\textsuperscript{-} concentrations decreased. Similar studies, involving careful analyses of pH, HCO\textsubscript{3}\textsuperscript{-}, and pCO\textsubscript{2} during perfusion of the colon of control and CLD patients also led Holmberg et al. (8) to the conclusion that apical Cl\textsuperscript{-}/HCO\textsubscript{3}\textsuperscript{-} exchange was absent in CLD patients. Finally, Jenkins and Milla (9) showed that Cl\textsuperscript{-} and HCO\textsubscript{3}\textsuperscript{-} fluxes in the rectum of CLD patients were driven by their electrochemical gradients and occurred independently of each other, in contrast to the coupled fluxes observed in normal controls, again consistent with an absence of apical Cl\textsuperscript{-}/HCO\textsubscript{3}\textsuperscript{-} exchange in the affected intestinal segments. The absence of apical Cl\textsuperscript{-}/HCO\textsubscript{3}\textsuperscript{-} exchange in ileum and colon of CLD patients (7–9), the demonstration that mutations in the DRA gene cause CLD (10, 11), the immunolocalization of DRA to the brush border in colonic epithelium (25), and our own data demonstrating that DRA is a Cl\textsuperscript{-}/HCO\textsubscript{3}\textsuperscript{-} exchanger, taken together, indicate that DRA is the major Cl\textsuperscript{-}/HCO\textsubscript{3}\textsuperscript{-} exchanger that mediates Cl\textsuperscript{-} absorption by ileal and colonic epithelial cells.

The modest up-regulation of DRA mRNA in the colon of mice lacking the NHE3 Na\textsuperscript{+}/H\textsuperscript{+} exchanger provides suggestive evidence that DRA is a component of the ion transport systems that limit the losses of fluid and electrolytes in a diarrheal state. The lack of NHE3 causes a severe intestinal absorptive defect, resulting in increased alkalinity, fluidity, and volume of the intestinal contents (18). The major compensatory mechanisms that have been identified so far are an increase in the size of each intestinal segment, along with massive up-regulation of the colonic H\textsuperscript{+},K\textsuperscript{+}-ATPase and increased activity of the epithelial Na\textsuperscript{+} channel in the colon (18). In colon, which is enlarged 3–fold in Nhe3\textsuperscript{−/−} mice, the activities of the H\textsuperscript{+},K\textsuperscript{+}-ATPase (42–44) and the Na\textsuperscript{+} channel (45, 46), along with that of an apical K\textsuperscript{+} channel, mediate the net exchange of extracellular Na\textsuperscript{+} and K\textsuperscript{+} for intracellular H\textsuperscript{+} and K\textsuperscript{+} and neutralizing luminal HCO\textsubscript{3}\textsuperscript{-}. However, the activities of these transporters alone are insufficient, as they do not provide a mechanism for absorbing Cl\textsuperscript{-}, and the amount of Cl\textsuperscript{-} that remains in the lumen limits the amount of Na\textsuperscript{+}, K\textsuperscript{+}, and fluid that can be absorbed. Also, because of the absorptive defect in the small intestine, the colon must absorb a greatly increased load of fluid and ions. By exchanging extracellular Cl\textsuperscript{-} and intracellular HCO\textsubscript{3}\textsuperscript{-}, DRA absorbs Cl\textsuperscript{-} directly, thereby enabling the other transporters to absorb Na\textsuperscript{+} and K\textsuperscript{+}.

The chloride, sulfate, and oxalate uptake activities of hDRA expressed in Xenopus oocytes have been shown to be inhibited by 1 mM DIDS (5, 12). In contrast, when Cl\textsuperscript{-} was removed from the extracellular medium containing HCO\textsubscript{3}\textsuperscript{-}, DIDS inhibited the alkalinization of mDRA-expressing HEK 293 cells by only 24 ± 3%. The observed difference in inhibitor sensitivity could be the result of the amino acid sequence divergence between mDRA and hDRA. Alternatively, the apparent difference in sensitivity could be due to differences in the expression system and/or experimental conditions. For example, the DIDS sensitivity of AE2 is much greater when expressed in Xenopus oocytes (39) than in HEK 293 cells (40). Also, the studies reported here measure only acute inhibition occurring immediately after addition of DIDS and not irreversible inhibition that might occur as a result of covalent modification of the transporter.
DRA Functions as a Cl⁻/HCO₃⁻ Exchanger

mediated Cl⁻ exchange. Cells were superfused with a Cl⁻-free, HCO₃⁻-containing solution during the time intervals indicated by the bar in the absence (left trace) and then in the presence (right trace) of 1 mM DIDS. This concentration of DIDS inhibited Cl⁻/HCO₃⁻ exchange by 24 ± 3% (n = 3 experiments). Lower panel, typical result testing the HCO₃⁻-dependence of mDRA-mediated anion exchange. Cells were superfused with a Cl⁻-free solution in the presence (left trace) and then in the absence (right trace) of HCO₃⁻. During the time intervals indicated by the bar, extracellular Cl⁻ was removed. The rate of alkalinization in HCO₃⁻-free solution was 13 ± 4% of the rate in the presence of HCO₃⁻ (n = 19 experiments).

directly and to absorb HCO₃⁻ indirectly via secretion of H⁺, with absorption of water following ion absorption. On the basis of these considerations, it seems likely that the absorption of ions and fluid in the colon of Nhe3⁻/⁻ mice is due primarily to the coupled activities of the DRA Cl⁻/HCO₃⁻ exchanger, the colonic H⁺,K⁺-ATPase, an apical K⁺ channel, and the epithelial Na⁺ channel. In wild-type mice additional absorptive capacity would be provided by the coupled activities of DRA and NHE3.

Northern blot analyses revealed increased expression of AE2 mRNA in both cecum and proximal colon of Nhe3⁻/⁻ mice, but not in distal colon where high levels of Na⁺ channel activity and colonic H⁺,K⁺-ATPase activity are likely to be the major contributors to the anion exchange process. These results suggest that DRA may be expressed at lower levels than DRA in all segments of the intestine. The role of AE2 in the intestine is unclear. It is conceivable that its expression is largely restricted to basolateral membranes where it would mediate HCO₃⁻ extrusion from the cell. If this is the case, then it would likely contribute to HCO₃⁻ absorption under conditions in which H⁺ secretion across the apical membrane exceeds the level of HCO₃⁻ secretion.

In conclusion, the experiments reported here show that DRA functions as a Cl⁻/HCO₃⁻ exchanger and raise the interesting possibility that it absorbs other anions besides Cl⁻ from the intestinal lumen. When the results of our experiments and the studies on human CLD patients (7–11) are considered together, they suggest that DRA is responsible for most, if not all, of the apical Cl⁻/HCO₃⁻ exchange in colon and possibly in ileum. Because of the absence of other Cl⁻ uptake mechanisms in colon, brush border membranes (15), DRA plays a pivotal role in the recovery of electrolytes and fluid from the intestinal lumen.

Fig. 4. DIDS sensitivity and HCO₃⁻-dependent anion exchange in cells expressing mDRA. HEK 293 cells were treated as described in Fig. 3. Upper panel, typical result testing the sensitivity of mDRA-mediated Cl⁻/HCO₃⁻ exchange to the anion exchange inhibitor DIDS. Cells were superfused with a Cl⁻-free, HCO₃⁻-containing solution during the time intervals indicated by the bar in the absence (left trace) and then in the presence (right trace) of 1 mM DIDS. This concentration of DIDS inhibited Cl⁻/HCO₃⁻ exchange by 24 ± 3% (n = 3 experiments). Lower panel, typical result testing the HCO₃⁻-dependence of mDRA-mediated anion exchange. Cells were superfused with a Cl⁻-free solution in the presence (left trace) and then in the absence (right trace) of HCO₃⁻. During the time intervals indicated by the bar, extracellular Cl⁻ was removed. The rate of alkalinization in HCO₃⁻-free solution was 13 ± 4% of the rate in the presence of HCO₃⁻ (n = 19 experiments).

Fig. 5. mDRA mediates electroneutral Na⁺-independent Cl⁻/HCO₃⁻ exchange. HEK 293 cells expressing mDRA were treated as shown in Fig. 3. Upper panel, to test the Na⁺ dependence of mDRA-mediated Cl⁻/HCO₃⁻ exchange, cells were superfused with a Cl⁻-free, HCO₃⁻-containing solution in the presence (left trace) and then in the absence (right trace) of extracellular Na⁺ during the time intervals indicated by the bar (n = 10 experiments). Lower panel, to determine whether mDRA-mediated Cl⁻/HCO₃⁻ exchange is electroneutral, cells were superfused with a Cl⁻-free, HCO₃⁻-containing solution with physiological extracellular K⁺ (5.4 mM; left trace) and then with high extracellular K⁺ (75 mM; right trace) to depolarize the membrane (n = 12 experiments). The different solutions were present during the time intervals indicated by the bars.

Fig. 6. Alterations in the expression of mDRA and AE2 mRNA in the intestine of wild-type and NHE3-deficient mice. Northern blot analysis of total RNA (10 µg/lane) from duodenum (Duod.), jejunum (Jejun.), ileum, cecum, proximal colon, and distal colon of wild-type (+/+1) and NHE3 homozygous mutant (+/−) mice was performed using an 0.4-kb DRA probe (top and middle panels). The blot was then stripped and analyzed using a 1.6-kb AE2 probe (bottom panel). The minor band below the 4.4-kb AE2 mRNA is residual 3.2-kb DRA mRNA that was resistant to the stripping procedure. Autoradiographic exposure times are shown on the right.

DRA 1 hr

DRA 7 hr

AE2 18 hr

Duod. Jejun. Ileum Cecum Prox. Colon Distal Colon

Cl Free Cl Free Cl Free Cl Free Cl Free Cl Free

HCO₃⁻ Free 1 mM DIDS HCO₃⁻ Free

Intracellular pH

Time (sec)

FIG. 4. DIDS sensitivity and HCO₃⁻-dependent anion exchange in cells expressing mDRA. HEK 293 cells were treated as described in Fig. 3. Upper panel, typical result testing the sensitivity of mDRA-mediated Cl⁻/HCO₃⁻ exchange to the anion exchange inhibitor DIDS. Cells were superfused with a Cl⁻-free, HCO₃⁻-containing solution during the time intervals indicated by the bar in the absence (left trace) and then in the presence (right trace) of 1 mM DIDS. This concentration of DIDS inhibited Cl⁻/HCO₃⁻ exchange by 24 ± 3% (n = 3 experiments). Lower panel, typical result testing the HCO₃⁻-dependence of mDRA-mediated anion exchange. Cells were superfused with a Cl⁻-free solution in the presence (left trace) and then in the absence (right trace) of HCO₃⁻. During the time intervals indicated by the bar, extracellular Cl⁻ was removed. The rate of alkalinization in HCO₃⁻-free solution was 13 ± 4% of the rate in the presence of HCO₃⁻ (n = 19 experiments).

FIG. 5. mDRA mediates electroneutral Na⁺-independent Cl⁻/HCO₃⁻ exchange. HEK 293 cells expressing mDRA were treated as shown in Fig. 3. Upper panel, to test the Na⁺ dependence of mDRA-mediated Cl⁻/HCO₃⁻ exchange, cells were superfused with a Cl⁻-free, HCO₃⁻-containing solution in the presence (left trace) and then in the absence (right trace) of extracellular Na⁺ during the time intervals indicated by the bar (n = 10 experiments). Lower panel, to determine whether mDRA-mediated Cl⁻/HCO₃⁻ exchange is electroneutral, cells were superfused with a Cl⁻-free, HCO₃⁻-containing solution with physiological extracellular K⁺ (5.4 mM; left trace) and then with high extracellular K⁺ (75 mM; right trace) to depolarize the membrane (n = 12 experiments). The different solutions were present during the time intervals indicated by the bars.

FIG. 6. Alterations in the expression of mDRA and AE2 mRNA in the intestine of wild-type and NHE3-deficient mice. Northern blot analysis of total RNA (10 µg/lane) from duodenum (Duod.), jejunum (Jejun.), ileum, cecum, proximal colon, and distal colon of wild-type (+/+1) and NHE3 homozygous mutant (+/−) mice was performed using an 0.4-kb DRA probe (top and middle panels). The blot was then stripped and analyzed using a 1.6-kb AE2 probe (bottom panel). The minor band below the 4.4-kb AE2 mRNA is residual 3.2-kb DRA mRNA that was resistant to the stripping procedure. Autoradiographic exposure times are shown on the right.
lumen in humans. It remains to be determined whether the role of DRA is as essential in other mammalian species as it is in humans, although its up-regulation in the colon of Nhe3−/− mice suggests that it is. Uncertainties arise from the relative absence of information about the role of the AE2 Cl−/HCO3− exchanger, which is present in at least low levels in brush border membranes of rabbit ileum (21) and is expressed throughout the intestinal tract of rat (20) and mouse (Fig. 6). It is possible that the primary intestinal function of AE2 is to mediate Cl−/HCO3− exchange across the basolateral membranes of cells that are engaged in net H+ secretion. Additional studies of the physiological functions and the biochemical and regulatory characteristics of both the DRA and the AE2 Cl−/HCO3− exchangers are needed to develop a better understanding of the ion transport mechanisms controlling absorption and secretion in the intestinal tract.

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