Regulation of DRA and AE1 in rat colon by dietary Na depletion

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Rajendran, Vazhaikkurichi M., Joel Black, Thomas A. Ardito, Pitchai Sangan, Seth L. Alper, Clifford Schweinfest, Michael Kashgarian, and Henry J. Binder. Regulation of DRA and AE1 in rat colon by dietary Na depletion. Am J Physiol Gastrointest Liver Physiol 279: G931–G942, 2000.—Two distinct Cl/anion exchange activities (Cl/HCO3 and Cl/OH) identified in apical membranes of rat distal colon are distributed in cell type-specific patterns. Cl/HCO3 exchange is expressed only in surface cells, whereas Cl/OH exchange is localized in surface and crypt cells. Dietary Na depletion substantially inhibits Cl/HCO3 but not Cl/OH exchange. We determined whether anion exchange isoforms (AE) and/or downregulated in adenoma (DRA) are expressed in and related to apical membrane anion exchanges by examining localization of AE isoform-specific and DRA mRNA expression in normal and Na-depleted rats. Amplification of AE cDNA fragments by RT-PCR with colonic mRNA as template indicates that AE1 and AE2 but not AE3 mRNAs are expressed. In situ hybridization study revealed that AE1 mRNA is expressed predominantly in surface but not crypt cells. In contrast, AE2 polypeptide is expressed in basolateral membranes and DRA protein is expressed in apical membranes of both surface and crypt cells. AE1 mRNA is only minimally present in proximal colon, and DRA mRNA abundance is similar in distal and proximal colon. Dietary Na depletion reduces AE1 mRNA abundance but does not alter DRA mRNA abundance. This indicates that AE1 encodes surface cell-specific aldosterone-regulated Cl/HCO3 exchange, whereas DRA encodes aldosterone-insensitive Cl/OH exchange.

surface cells; crypt cells; in situ hybridization; immunohistochemistry; Cl/anion exchange

Electroneutral Na-Cl absorption is the result of parallel Na/H exchange (NHE) and Cl/HCO3 exchanges thought to be coupled via intracellular pH (pHi) (5, 6). Recent molecular studies have identified the expression of three different NHE isoforms (NHE1, NHE2, and NHE3) in epithelial cells of rat distal colon (7, 8, 17). These studies also demonstrated that NHE2 and NHE3 isoform-specific proteins are expressed in apical membranes, whereas NHE1 protein is localized to basolateral membranes (7, 8, 17). On the basis of the differential regulation of NHE isoforms by mineralocorticoids, it has been proposed that NHE2 and NHE3 isoforms are responsible for Na absorption, whereas NHE1 is responsible for pHi homeostasis (17).

Two distinct Cl/anion exchange activities (i.e., Cl/HCO3 and Cl/OH exchanges) have been identified in apical membrane vesicles (AMV) isolated from rat distal colon (30). Recent studies have established that Cl/HCO3 exchange is responsible for vectorial transport of Cl and HCO3 ions, whereas Cl/OH exchange may be responsible for the regulation of intracellular functions such as pHi and volume (31). These conclusions are supported by several observations: 1) Cl/HCO3 exchange is expressed only in surface (absorptive) and not in crypt (secretory) cells; 2) Cl/OH exchange is expressed in both surface and crypt cells (31); and 3) dietary Na depletion, which reduces net Cl absorption (44), also substantially inhibits Cl/HCO3 exchange without affecting Cl/OH exchange (31).

Cl/HCO3 exchange function has been linked to three anion exchange (AE) cDNAs (i.e., AE1, AE2, and AE3) in noncolonic tissues (1, 20) and to downregulated in adenoma (DRA) cDNA in human and mouse colon (19, 27). Although AE cDNAs have been cloned from one or more rat tissues (21, 22), their role in Cl/anion exchange in the rat colon has not been established. In contrast, recent observations in patients with congenital chloride diarrhea indicate that DRA most likely encodes Cl/HCO3 exchange in both colon and ileum of humans and that a DRA cDNA cloned from mouse colon expresses Cl/OH and Cl/HCO3 exchange function in HEK cells (14, 19, 27).

The present study was designed to identify whether AE isoforms or DRA encode the anion exchanges iden-
tified in apical membranes of rat distal colon. Since Na depletion and aldosterone markedly inhibit active Cl absorption and Cl/HCO₃ exchange in rat distal colon (31, 44), we studied whether aldosterone-induced changes in Cl/anion exchanges could be correlated with modification of either AE isoform-specific or DRA mRNA abundance. These observations suggest the possibility that the aldosterone-sensitive fraction of Cl/HCO₃ exchange and vectorial Cl absorption is not encoded by DRA but by AE1 isoform, whereas the aldosterone-insensitive component is encoded by DRA.

MATERIALS AND METHODS

mRNA isolation. Total RNA and mRNA from colonicocytes were isolated from the distal colon of both normal and Na-depleted rats (Sprague Dawley, 200–250 g) by the method of Sambrook et al. (36), as previously described (17). Total RNA and mRNA were also isolated from normal kidney. In brief, total RNA from isolated colonicocytes and whole kidney homogenized in 4 M guanidine isothiocyanate was isolated by CsCl centrifugation. mRNA was purified using an oligo(dT)-cellulose column (Boehringer Mannheim, Indianapolis, IN). Colonicocytes isolated from two colons were used for total RNA preparation. mRNA was purified from at least three different RNA preparations. All solutions were prepared in diethyl pyrocarbonate-treated autoclaved water.

cDNA probes. AE1, AE2, and AE3 isoform-specific cDNA fragments were amplified using RT-PCR techniques (Clontech, Palo Alto, CA). For RT-PCR, mRNA isolated from rat distal colon was used as template, and AE isoform-specific nucleotide primers (20-mers) were designed from published sequences (21, 22). Isoform-specific primers were designed at the regions of translated amino acid sequences with relatively low homology between isoforms. Isoform-specific primers used were: AE1, sense 5′-GCTGAGGACCTAAAGGA-3′ and antisense 5′-TCCTTTCCCGCTCTAAATGC-3′ that span nucleotides 742–1349 of rat kidney AE1 cDNA (9); AE2, sense 5′-AGCTGACCTGCCACCTCTA-3′ and antisense 5′-TCCTTTCCCGCTCTAAATGC-3′ that span nucleotides 1482–2201 of rat stomach AE2 cDNA (21); and AE3, sense 5′-GATGACAGGAAGGTGGTT-3′ and antisense 5′-TCTTCAGAGGTGGCTCGGA-3′ that span nucleotides 1319–1728 of rat brain AE3 cDNA (21). All sense and antisense primers were appended with BamH I and EcoR I restriction sites, respectively. One microgram of distal colonic mRNA was reverse transcribed using oligo(dT) and random primers and Moloney murine leukemia virus reverse transcriptase (Clontech). Thirty-cycle PCR reaction was carried out in a DNA thermal cycler 2400 (Perkin-Elmer, Norwalk, CT) with denaturation at 94°C for 30 s, annealing at 55°C for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 5 min. PCR products were analyzed by agarose gel electrophoresis. Expected-size cDNA fragments were seen for AE1 (Fig. 1A) and AE2 (Fig. 1B) but not for AE3 isoforms. Expected-size cDNA fragments were extracted using a gel extraction kit (Qiagen, Chatsworth, CA).

Subcloning. PCR products were ligated into either pUC19 (New England Biolabs, Beverly, MA) or pBluescript II SK (Stratagene, Palo Alto, CA) vectors that were digested with BamH I and EcoR I. cDNA-ligated plasmid was transformed into Escherichia coli (XL1-Blue) using a Gene Pulse II electroporator (Bio-Rad, Hercules, CA). Plasmids with expected-size inserts were selected and sequenced using an automated Applied Biosystems 373A fluorescent sequencer (W. M. Keck Biotechnology Resource Facility, Yale University School of Medicine, New Haven, CT). Nucleotide sequences were analyzed by computation at the National Center for Biotechnology Information using BLAST network service. Specificity of the cDNA fragments were tested by Southern blot analyses using full-length cDNAs of AE1, AE2, and AE3 isoforms (generously provided by Dr. Gary E. Shull, University of Cincinnati, Cincinnati, OH). The RT-PCR-amplified colonic AE cDNA fragments hybridized only with the respective AE cDNAs without any cross-hybridization, indicating that these cDNA fragments are isoform specific (data not shown).

In this study, full-length coding sequence of human DRA cDNA was used as a probe to analyze mRNA of rat distal colon (28); DRA cDNA has been cloned and shown to be expressed predominantly in the colon of both human and mouse (27, 37). This full-length human DRA cDNA was kindly provided by Richard Moseley (University of Michigan, Ann Arbor, MI) (28).

Northern blot. Five micrograms of mRNA electrophoresed on a 1% formaldehyde-agarose gel was transferred to nylon
membrane (NEN, Boston, MA) by capillary action using 20× standard saline citrate (SSC) solution. mRNA linked to membranes using UV-Stratallinker 2400 (Stratagene) was exposed for 2 h with prehybridization solution (50% deionized formamide, 10% dextran sulfate, 1 M NaCl, and 1% SDS) at 42°C in a HyRoller hybridization oven (Owl Scientific, Woburn, MA). Random primer-labeled \[^{[32}P\]cDNA probe (1–2 × 10^5 cpm/ml) (Boehringer Mannheim) was added and hybridized at 42°C for 18 h. Blots were washed twice with 2× SSC for 5 min at room temperature. Following a wash with 2× SSC containing 0.1% SDS for 30 min at 45°C, blots were exposed for autoradiography with Hyper film (Amersham, Little Chalfont, UK). Autoradiographs were incubated at −70°C and developed after 18 h exposure. Blots were stripped by boiling in 0.1× SSC-0.5% SDS solution for 5 min and hybridized sequentially with AE1, AE2, DRA, and glyceraldehyde 3-phosphate dehydrogenase probes. Quantitatively identical results were observed in at least three different blots. mRNA abundances were quantitated with Personal Densitometer SI using ImageQuant software.

**Western blot analysis.** One hundred milligrams of membrane proteins were resolved by SDS-PAGE on a 10% gel using a Bio-Rad Mini-protein II electrophoresis apparatus, as described earlier (34). Proteins were transferred to nitrocellulose filter (Biorad; Gelman Sciences, Ann Arbor, MI) at 200 mA for 60 min at 4°C in 192 mM glycine, 25 mM Tris (pH 8.3), and 20% methanol. Filters were then incubated in 20 mM Tris (pH 7.5), 137 mM NaCl, and 0.1% Tween 20 containing nonfat dry milk (TBST) for 1 h at room temperature to block any nonspecific sites in the membrane filter. Immunostaining was performed in TBST containing 0.25 μg/ml of DRA antibody (11) overnight at 4°C. Filter was then washed three times for 15 min/wash in 50 ml of TBST. The filter was then incubated with secondary antibody, ant-rabbit IgG horseradish peroxidase conjugate (1:5,000 dilution), for 1 h at room temperature. Filter was then washed three times for 15 min/wash, and finally antibody-specific protein band was visualized by enhanced chemiluminescence (Amersham, Chicago, IL).

**Immunohistochemical studies.** Rats were perfused with paraformaldehyde-lysine-periodate (PLP) fixative for 2 min. Colon was removed and dissected into millimeter-sized pieces, which were postfixified for 1 h at room temperature in PLP. The fixation was followed by three 15-min washes in PBS. Tissue was then cryoprotected in PBS with 10% DMSO for 10 min and frozen in individual pieces under swirling freon-22, which was undercooled to approximately −100°C with liquid nitrogen. Tissue was stored under liquid nitrogen until use.

Tissue was cut on a Leica CM-3000 cryostat (Nessloch, Germany) at 6-μm thickness and picked up on lysine-coated slides. Sections were then rehydrated in PBS and blocked with PBS with 1% BSA-1% normal goat serum for 1 h. DRA antibody (1:100) (11) was used in a standard indirect immunofluorescence protocol with appropriate TRITC-conjugated secondary antibody (Zymed, San Francisco, CA). Studies with AE2 polyclonal antibody (1:1,000) (2) employed a biotinylated amplification method. Thus tissue was blocked using an avidin-biotin blocking kit (Vector Laboratories, Burlingame, CA) when needed. General labeling procedures and washing and incubation times for both labeling methods were similar. Briefly, sections were incubated with primary antibody for 1 h at room temperature, washed three times for 10 min each in 0.1% BSA in PBS, and then exposed to secondary TRITC conjugate of biotinylated secondary antibody as required and washed as above. AE2-labeled sections were then exposed to streptavidin-conjugated fluorochrome and again washed. Sections were then overslipped with Vectashield antifade medium (Vector) and edge-sealed with Cytoseal-60 Mounting medium (Stephen’s Scientific, Riverdale, NJ). Slides were stored at 4°C until viewing.

**Confocal microscopy.** Confocal microscopy was performed with a Bio-Rad MRC-600 scanning head attached to an Olympus-B upright microscope equipped with a 60× oil immersion lens. All microscope settings controlling laser inten-
sity, detection sensitivity, and confocal aperture size were standardized to ensure optimal signal-to-noise ratio. Negative control slides and labeled tissue were then viewed under the same settings.

RESULTS

A preliminary study was designed to identify the expression of existing AE isoforms in rat distal colonic epithelial cells. In this study, mRNA isolated from colonic epithelial cells was used as template to amplify AE isoform-specific cDNA fragments by RT-PCR. Expected-size cDNA fragments were amplified for AE1 (Fig. 1A) and AE2 (Fig. 1B) but not for AE3 isoforms (data not shown). Nucleotide sequence of the RT-PCR-amplified AE1 and AE2 cDNA fragments confirmed their identities (data not shown). This observation provided the initial demonstration of the expression of at least two AE gene products in the rat distal colon. The following studies utilized the AE1 and AE2 isoform-specific cDNA fragments as probes to establish the expression of AE isoforms in rat distal colon. Northern blot analyses of DRA were also performed, using the full-length coding sequence of the human cDNA (28) as a probe. We did not amplify a DRA fragment by RT-PCR because DRA has been cloned and shown to be expressed predominantly in the colon of both human and mouse (27, 37).

mRNA expression. Northern blot analyses were performed on mRNA isolated from epithelial cells of normal rat distal colon to establish the expression of DRA and AE isoforms. mRNA isolated from rat kidney was also used in the Northern blot analyses because a rat epithelial AE1 was first cloned from kidney (22). As shown in Fig. 2A, DRA cDNA hybridized with a 3.4-kb mRNA in distal colon but not in kidney. As shown in Fig. 2B, the AE1 cDNA probe hybridized with a 4.6-kb mRNA in both distal colon and kidney. In addition to 4.6-kb mRNA, the AE1 probe also hybridized with 2.3-kb mRNA in kidney and distal colon. This 2.3-kb transcript could represent either an alternatively spliced AE1 isoform or nonspecific binding (Fig. 2B). The AE2 cDNA probe also hybridized with a 4.4-kb mRNA in both colon and kidney (Fig. 2C). Because we were not able to amplify an AE3 isoform-specific cDNA fragment by RT-PCR, we used both brain- and heart-specificfull-length AE3 cDNAs as probes to examine the expression of AE3 isoform-specific mRNA (25). AE3 isoform-specific cDNA probes did not hybridize with mRNA isolated from colonocytes of rat distal colon (data not shown). These results indicate that DRA, AE1, and AE2 isoform-specific mRNAs are expressed in colonocytes of normal rat distal colon.

The demonstration that specific Na/H exchange isoforms are expressed both in different types of colonocytes and in different membranes of the same colonocyte suggested that these isoforms might perform different cell functions, e.g., absorption and pHi regulation (43). Although both AE1 and AE2 mRNAs are expressed in rat distal colonocytes, it is not known

![Fig. 2. Expression of downregulated in adenoma (DRA; A), AE1 (B), and AE2 (C) isoform-specific mRNA. mRNA isolated from proximal colon, distal colon, and kidney of normal rats was analyzed by Northern blot hybridization using respective cDNA probes, as described in MATERIALS AND METHODS. mRNA positions and sizes (in kb) are shown at left. Northern blot was also probed with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal control.](http://ajpgi.physiology.org/)
whether both isoforms are expressed in the same and/or different cell types. Thus in situ hybridization studies were performed to localize AE1 isoform-specific mRNA expression in rat distal colon. As shown in Fig. 3A, AE1 antisense hybridization signal was present within surface cells but not within crypt cells of the distal colon. In control experiments, AE1 hybridization product was localized to probable intercalated cells of the kidney (Fig. 3E), consistent with prior studies (1, 15, 38). No hybridization signal was observed with AE1 sense probe in either distal colon (Fig. 3B) or kidney (Fig. 3F). In contrast to AE1, AE2 hybridization signal was detected in both surface and crypt cells of the distal colon (Fig. 4). These results suggest the possibility that AE1 gene may encode an anion exchange responsible for Cl absorption because absorptive processes are generally believed to be present in surface but not in crypt cells (6).

Protein localization. Immunofluorescent studies with DRA and AE2 antibodies were performed to establish their cell- and membrane-specific localization in rat distal colon (Figs. 5 and 6). As shown in Fig. 5, DRA antibody localized protein expressed exclusively in apical membranes of surface cells and at lower intensity in the upper 50% of crypt cells. No evidence of basolateral membrane localization was seen. In contrast, AE2 antibody detects a protein that is expressed only in basolateral membrane of surface cells (Fig. 6). AE2 antibody localizes proteins that are expressed in both basolateral membranes and cytosolic granules in crypt cells (Fig. 6D). Control serum did not localize any protein in colonic epithelial cells (Fig. 6A), but AE2 immunostaining was blocked by peptide antigen (Fig. 6C).

In view of the known effect of Na depletion on and the segmental differences in Cl absorption, further experiments were designed. We examined 1) the effect of dietary Na depletion (aldosterone) and 2) the tissue specificity of expression (i.e., proximal vs. distal colonic segments) of DRA and AE1 mRNA abundances and of Cl/anion exchange activities.

Effect of Na depletion. The coupling of Na/H and Cl/HCO₃ exchanges is the mechanism for electroneutral Na-Cl absorption in rat distal colon (5, 6, 30, 32).

Fig. 3. Cell-specific expression of AE1 mRNA. A: AE1 hybridization signal is present in surface cells (arrowheads) but not in crypt cells (asterisks) of normal rat distal colon. B: no hybridization signal is present in surface cells or crypt cells of distal colon probed with AE1 sense riboprobe. C: surface cells and crypt cells of proximal colon do not exhibit AE1 hybridization signal. D: no hybridization signal is present in proximal colon when probed with AE1 sense riboprobe. E: intercalated cells (arrowheads) of the kidney exhibit AE1 hybridization signal. F: no hybridization signal is present in kidney when probed with AE1 sense riboprobe. Magnification, × 275; bar, 50 μm.
Dietary Na depletion substantially inhibits Na-Cl absorption, Na/H exchange, and Cl/HCO₃ exchange and reduces NHE2 and NHE3 isoform-specific mRNA abundances and protein expression in rat distal colon (17, 31, 32). It is not known whether Na depletion also alters DRA and AE1 mRNA abundance. Thus Northern blot analyses of mRNA isolated from Na-depleted rats were performed with DRA- (Fig. 7A), AE1- (Fig. 9A) and AE2- (Fig. 10A) specific cDNA probes. As shown in Fig. 7B, Na depletion did not alter DRA mRNA abundance. Similarly, DRA protein expression was not altered by dietary Na depletion in apical membranes isolated from surface cells of distal colon (Fig. 8). In contrast, Na depletion reduced the AE1 isoform-specific mRNA abundance by 86% (Fig. 9B) but did not alter the AE2 mRNA abundance (Fig. 10B). These results are consistent with the hypothesis that DRA may encode aldosterone-insensitive Cl/anion exchange and AE1 may encode aldosterone-sensitive Cl/HCO₃ exchange in rat distal colon.

Segmental distribution. Significant qualitative differences exist in the characteristics of Na and Cl absorption in proximal and distal segments of the rat colon, as shown by ion flux studies performed across isolated intact mucosa under voltage clamp conditions (5, 12). Prior studies of Cl uptake using AMV prepared from surface cells of distal colon revealed separate and distinct Cl/HCO₃ and Cl/OH exchanges (30). To provide additional characterization of anion exchanges in the large intestine, studies were performed in proximal colon.

As shown in Fig. 11, both Cl/HCO₃ and Cl/OH exchanges were identified in AMV prepared from surface cells of proximal colon. Cl/HCO₃ exchange activity was 0.3 ± 0.1 nmol·mg protein⁻¹·12 s (Fig. 11A) and was ~6% of Cl/OH exchange (4.9 ± 1.1 nmol·mg protein⁻¹·12 s) (Fig. 11B). In contrast, in AMV prepared from surface cells of distal colon, Cl/HCO₃ and Cl/OH exchange activities were 1.4 ± 0.2 and 5.8 ± 0.8 nmol·mg protein⁻¹·12 s, respectively (Ref. 30 and Fig. 11B).

Fig. 4. Cell-specific expression and localization of AE2 mRNA. A: AE2 hybridization signal is detected in both surface (arrowheads) and crypt (asterisks) cells of the distal colon. B: no hybridization signal is present in surface or crypt cells of distal colon probed with AE2 sense riboprobe. Magnification, ×275; bar, 50 μm.

Fig. 5. Immunohistochemical localization of DRA protein in distal colon. Colonic tissue was fixed in paraformaldehyde-lysine-periodate (PLP) and processed as described in MATERIALS AND METHODS. DRA protein is localized to apical membrane of surface cells and upper 50% of crypt cells (B). No labeling was seen in the absence of antibody (A). Antibody was diluted 1:100. Magnification, ×500.
As a consequence, Cl/HCO₃ exchange activity in proximal colon was 21% of its activity in distal colon (Fig. 11A), whereas Cl/OH exchange activity in AMV prepared from surface cells of proximal colon was 84% of that in AMV from distal colon (Fig. 11B). These studies indicate that Cl/HCO₃ exchange activity in proximal colon is substantially less than its activity in distal colon, whereas Cl/OH exchange activities were relatively comparable in both proximal and distal colon.

Studies of DRA and AE1 expression were also performed in proximal colon. DRA mRNA and protein expression were determined in proximal colon. DRA mRNA levels in proximal and distal colon were equivalent (Fig. 2A). Similar to its mRNA abundance, DRA protein in Western blot analyses was comparable in apical membranes isolated from both proximal and distal colon (Fig. 8). In contrast, AE1 mRNA abundance was minimally expressed in proximal colon (Fig. 2B). To confirm the minimal expression of AE1 mRNA in proximal colon, in situ hybridization studies were performed, as shown in Fig. 3C. AE1 antisense hybridization was not present in proximal colon of both surface and crypt cells (Fig. 3C); AE1 sense hybridization was also not identified (Fig. 3D). These results provide additional correlation between the AE1 expression and Cl/HCO₃ exchange activity and between DRA expression and Cl/HCO₃ exchange activity.
ion and Cl/OH exchange activity (Table 1), supporting the thesis that AE1 encodes Cl/HCO₃ exchange and DRA encodes Cl/OH exchange.

DISCUSSION

Electroneutral Na-Cl absorption in the rat distal colon is the result of the functionally coupled action of Na/H and Cl/HCO₃ exchanges (5, 6). Aldosterone and Na depletion inhibit electroneutral Na-Cl absorption in the distal colon by inhibition of both Na/H and Cl/HCO₃ exchanges (17, 31, 32). Although several genes capable of expressing anion exchange functions have been cloned (1, 20), the gene responsible for aldosterone regulation of Cl absorption in the distal colon has not been identified.

To date, three different AE genes have been identified in one or more noncolonic tissues (1, 20). Using in vitro expression studies, AE1, AE2, and AE3 polypeptides have each been shown to be responsible for Cl/HCO₃ exchange activity (1, 3, 16, 20, 24, 29, 41). Each AE isoform has a tissue- and membrane-specific expression pattern (1, 20, 21). In addition, 5’ variant transcripts of both AE1 and AE2 have also been described (23, 45).

Another gene that regulates Cl/HCO₃ exchange is DRA (27, 28). DRA was initially identified in normal human colon but was absent in many patients with adenocarcinoma (37). Its role in the adenoma-to-carcinoma sequence is not known, although DRA may be a marker of cell differentiation (19). In contrast, DRA has been identified as the gene whose mutation leads to congenital chloride diarrhea (or congenital chloride diarrhea), which is characterized by diarrhea and an elevated stool Cl concentration (14). Luminal perfusion studies in patients with congenital chloride diarrhea indicate an absence of Cl absorption and HCO₃ secretion in the ileum and colon, suggesting that DRA may function as an apical membrane Cl/HCO₃ exchanger (14). Subsequent studies with DRA cRNA expressed in oocytes demonstrated the expression of Cl and sulfate uptake but could not distinguish between Cl/HCO₃ and Cl/OH exchanges (28, 39). A recent communication reported the cloning of DRA from mouse colon (mDRA) with 81% identity to that of human DRA. Expression studies in HEK cells transfected with mDRA provided evidence of both Na-independent Cl/OH and Cl/HCO₃ exchanges and concluded that mDRA encoded the colonic Cl/HCO₃ exchange and was responsible for Cl absorption (27).

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Fig. 8. DRA protein expression. Western blot analysis was performed as described in MATERIALS AND METHODS using DRA antibody at a concentration of 0.25 μg/ml. A protein of ~90 kDa was identified in apical membranes from normal distal colon (lane 1), Na-depleted distal colon (lane 2), and normal proximal colon (lane 4) but not in basolateral membranes from normal distal colon (lane 3). Arrow indicates DRA protein band.

Fig. 9. Regulation of AE1 isoform-specific mRNA abundance in rat distal colon. A: mRNA isolated from epithelial cells of normal and Na-depleted rat distal colon was analyzed by Northern blot hybridization using AE1 isoform-specific cDNA probe, as described in MATERIALS AND METHODS. Northern blot was also analyzed with GAPDH as an internal control. mRNA positions and sizes (in kb) are shown at left. B: mRNA abundances in normal (open bar) and Na-depleted (closed bar) rats were quantitated by densitometry from 3 different mRNA preparations. Arbitrary units presented were normalized to GAPDH mRNA abundance.
The present studies establish the presence of mRNA encoding DRA, AE1, and AE2 in epithelial cells of rat distal colon (Figs. 1 and 2). Although a full-length AE isoform-specific cDNA has not been cloned from colonic epithelial cells, RT-PCR-amplified cDNA fragments have 100% nucleotide identity with existing AE1 and AE2 isoform cDNAs (Fig. 1). These RT-PCR-derived cDNA fragments were then used as probes to establish the expression of AE1 and AE2 isoform-specific mRNAs in rat distal colon. Specific AE1 and AE2 cDNA probes hybridized with mRNAs of indistinguishable size in both kidney and distal colon (Fig. 2, B and C).

Membrane localization studies were performed with a polyclonal antibody to AE2 isoform. Immunohistochemistry analyses with this antibody established the presence of AE2 isoform protein to the basolateral membrane (Fig. 6). Although prior studies had failed to identify Cl-anion transport processes in basolateral membrane of rat distal colon, Cl/HCO_3^- and Cl/OH exchange function has recently been demonstrated in these membranes (18). These observations are not unexpected, because AE2 polypeptide has been identified in basolateral membranes of mouse colon surface cells (2) and both AE2 and Cl/HCO_3^- exchange activity have been identified in rabbit ileal basolateral membranes (35). Finally, since aldosterone did not affect AE2 isoform mRNA abundance or inhibit Cl/HCO_3^- exchange activity in BLMV (unpublished observations), it is likely that AE2 isoform encodes Cl/HCO_3^- (and perhaps Cl/OH) exchange functions in the basolateral membranes of rat distal colon.

Previous studies have demonstrated the existence of two distinct anion exchangers capable of transporting Cl in AMV of rat distal colon (Cl/HCO_3^- and Cl/OH exchanges) (30, 31). Several observations in the present study suggest correlations between regulation of mRNA levels and Cl/anion exchange activities (Table 1). First, both AE1 isoform-specific mRNA expression (Fig. 3A) and Cl/HCO_3^- exchange activity (31) are present only in surface and not in crypt cells. Second, AE1 mRNA level (Fig. 9B) and Cl/HCO_3^- exchange activity (31) are reduced by 86% and 67%, respectively, in Na-depleted animals. Third, Na depletion did not alter DRA mRNA or protein expression (Figs. 7 and 8) or Cl/OH exchange activity (31). Fourth, AE1 mRNA expression (Fig. 3C) and Cl/HCO_3^- exchange were absent or minimally present, respectively, in proximal colon (Fig. 11), but DRA mRNA abundance and Cl/OH exchange activity are both present in proximal colon (Figs. 2A and 11).

Similar to the distinct spatial distribution of Cl/HCO_3^- and Cl/OH exchange activities (31), the localization of AE1 and DRA mRNAs differs in surface and crypt cells. AE1 mRNA is detectable in surface but not in crypt cells of the distal colon (Fig. 3A), whereas DRA mRNA was evenly distributed throughout the distal colon in prior studies with human colon (11). The present study revealed the presence of DRA protein in apical membranes of surface and the upper 50% of crypt cells (Fig. 5), which is in agreement with a previous report in human colon (11). This pattern of AE1 isoform and DRA mRNA distribution in surface and crypt cells parallels the distribution of Cl/HCO_3^- exchange and Cl/OH exchange activities, respectively, in AMV from distal colon (31).

As previously noted, recent studies show that DRA can mediate Cl/OH and Cl/HCO_3^- exchanges (14, 27). The present study confirms the expression of DRA mRNA in the rat distal and proximal colon (Fig. 2A), similar to its known presence in human and mouse colon (27, 37). DRA mRNA and protein levels were not
altered in the distal colon of Na-depleted rats (Figs. 7 and 8), in contrast to the inhibition of Cl/HCO₃ exchange (31) and AE1 mRNA (Fig. 9B) by Na depletion. However, the partial inhibition by aldosterone of both active Cl absorption (73%) (44) and Cl/HCO₃ exchange (67%) (31) indicates the presence of two distinct components of Cl transport, one that is aldosterone sensitive and another that is insensitive to aldosterone. Since dietary Na depletion only partially inhibits Cl/HCO₃ exchange (31) and has differential effects on levels of DRA and AE1 mRNAs, it is interesting to speculate that an AE1 gene product might encode the aldosterone-sensitive Cl/HCO₃ exchange and that DRA encodes aldosterone-insensitive Cl/OH exchange, which also transports HCO₃ (31).

The relative contribution of AE1 and DRA to overall apical membrane Cl/HCO₃ exchange and overall Cl absorption may vary in the large intestine of different species. The observations in patients with congenital chloride diarrhea suggest that in humans DRA controls the predominant fraction, if not all, of colonic Cl absorption (19). In contrast, in the rat distal colon several observations indicate that a DRA-independent pathway, perhaps AE1, may mediate the major fraction of active colonic Cl absorption and apical membrane Cl/HCO₃ exchange. First, aldosterone inhibits ~80% of active Cl absorption when studied under voltage clamp conditions (44), Cl/HCO₃ exchange in studies of Cl uptake in AMV (31), and AE1 mRNA (Fig. 9). Since neither DRA mRNA nor protein is altered by dietary Na depletion (Fig. 7 and 8), the 20% residual active Cl absorption and Cl/HCO₃ exchange remaining in the distal colon of Na-depleted rats may represent the component of colonic Cl transport that is regulated by DRA. Although DRA has been cloned from the mouse colon and expressed as both Cl/HCO₃ and Cl/OH exchanges in HEK cells (27), there is insufficient information available about Cl transport and Cl/HCO₃ exchange in the mouse colon to determine whether DRA regulates a small or major component of Cl absorption and Cl/HCO₃ exchange.

Understanding of the membrane-specific localization of AE isoforms is presently incomplete in polarized epithelia. The expression of AE1 isoform protein has been localized to basolateral membranes of acid-secreting intercalated cells of cortical and medullary collecting ducts (38). Although studies in native tissue clearly indicate the presence of anion exchange function in apical membranes, unequivocal anion exchange localization in apical membranes in mammals in situ remains undemonstrated. AE2 protein has previously been expressed in basolateral membranes of rat stomach and intestine, mouse colon and intestine, and human intestine (2, 3, 10, 35, 42). The present studies confirm the localization of AE2 isoform-specific protein to the basolateral membrane of rat distal colon (Fig. 6).

In conclusion, the present studies demonstrate the differential expression of AE1 and AE2 mRNA in rat distal colon and the differential effects of Na depletion on both AE and DRA mRNAs. AE2 polypeptide likely mediates anion exchange in basolateral membranes of both surface and crypt cells, whereas DRA likely me-

Table 1. Comparison between AE1 and DRA mRNA abundance and Cl/anion function: importance of colonic segment, cell type, and dietary Na depletion

<table>
<thead>
<tr>
<th>Segment</th>
<th>Cell Type</th>
<th>AE1 mRNA</th>
<th>AMV Cl/HCO₃ Exchange</th>
<th>DRA mRNA</th>
<th>AMV Cl/OH Exchange</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distal, normal</td>
<td>Surface cell</td>
<td>4+</td>
<td>4+ (31)</td>
<td>4+</td>
<td>4+ (31)</td>
</tr>
<tr>
<td>Distal, normal</td>
<td>Crypt cell</td>
<td>0</td>
<td>&lt;1+ (31)</td>
<td>3+ (10)</td>
<td>2+ (31)</td>
</tr>
<tr>
<td>Distal, Na-depleted</td>
<td>Surface cell</td>
<td>1+</td>
<td>1+ (31)</td>
<td>4+</td>
<td>4+ (31)</td>
</tr>
<tr>
<td>Proximal, normal</td>
<td>Surface cell</td>
<td>0</td>
<td>1+</td>
<td>4+</td>
<td>4+</td>
</tr>
</tbody>
</table>

Semiquantitative assessment of mRNA abundance and Cl/HCO₃ and Cl/OH exchange activity is presented in arbitrary units. Numbers in parentheses are references from which data was taken. AE, anion exchanger; DRA, downregulated in adenoma; AMV, apical membrane vesicles.
diates the apical membrane Cl/anion exchange that is insensitive to Na depletion (aldosterone). The correlations established in this paper suggest the hypothesis that an AE1 gene product may mediate aldosterone-sensitive (dietary Na depleted-sensitive) Cl/HCO3 exchange of apical membranes in surface cells of rat distal colon.

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REFERENCES


