The distribution and regulation of apical Cl/anion exchanges in surface and crypt cells of rat distal colon

Rajendran, Vazhaikkurichi M., and Henry J. Binder.

Distribution and regulation of apical Cl/anion exchanges in surface and crypt cells of rat distal colon. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G132–G137, 1999.—Na depletion inhibits electroneutral Na-Cl absorption in intact tissues and Na/H exchange in apical membrane vesicles (AMV) of rat distal colon. Two anion (Cl/HCO3 and Cl/OH) exchanges have been identified in AMV from surface cells of rat distal colon. To determine whether Cl/HCO3 and/or Cl/OH exchange is responsible for vectorial Cl movement, this study examined the spatial distribution and the effect of Na depletion on anion-dependent 36Cl uptake by AMV in rat distal colon. These studies demonstrate that HCO3 concentration gradient-driven 36Cl uptake (i.e., Cl/HCO3 exchange) is primarily present in AMV from surface cells and 2) markedly reduced by Na depletion. In contrast, OH concentration gradient-driven 36Cl uptake (i.e., Cl/OH exchange) present in both surface and crypt cells is not affected by Na depletion. In Na-depleted animals HCO3 also stimulates 36Cl via Cl/OH exchange with low affinity. These results suggest that Cl/HCO3 exchange is responsible for vectorial Cl absorption, whereas Cl/OH exchange is involved in cell volume and/or cell pH homeostasis.

**Electroneutral Chloride Absorption**

Electroneutral chloride absorption is both Na and HCO3 dependent and is the primary mechanism of Cl absorption in the rat distal colon (4). On the basis of studies in both intact tissue and apical membrane vesicles (AMV), the general consensus has been that electroneutral Na-Cl absorption is the result of the coupling of Na/H exchange and Cl/HCO3 exchange (3, 9–11, 14, 15).

Previous studies (15) of Cl/anion exchange concluded that Cl/HCO3 and Cl/OH exchanges are distinct and separate anion exchanges. This conclusion was based on several observations. First, in the absence of HCO3, OH concentration ([OH]) gradient-driven 36Cl uptake yielded a sigmoidal curve as a function of [OH] consistent with the participation of two Cl transport systems on these apical membranes (15). Second, in the presence of HCO3, [OH] gradient-driven 36Cl uptake was hyperbolic as a function of [OH] (15). Taken together, these two findings suggested that the [OH] gradient stimulates 36Cl uptake via both Cl/OH and Cl/HCO3 exchanges, while the HCO3 concentration ([HCO3]) gradient stimulates 36Cl uptake only via Cl/HCO3 exchange. Third, there was a 20-fold difference in the inhibitor constant (Kn) for DIDS for Cl/OH and Cl/HCO3 exchanges (15). Fourth, the Michaelis constant (Km) for Cl for these two Cl/anion exchanges also significantly differed (15).

Na depletion inhibits electroneutral Na-Cl absorption as well as stimulating electrogenic Na absorption in rat distal colon (4, 10, 12). Studies with AMV isolated from normal and Na-depleted animals demonstrated that Na depletion both inhibited Na and Cl uptake (i.e., Na/H exchange) and induced amiloride-sensitive apical Na channels (17). Because preliminary studies (18) demonstrated that Na depletion partially inhibited Cl/anion exchange in AMV from rat distal colon, these present experiments were designed to determine whether Na depletion altered Cl/HCO3 and/or Cl/OH exchanges.

The present study was initiated to identify the colonic Cl/anion exchange activity responsible for vectorial Cl movement. In this study, the spatial distribution in surface and crypt cells and the Na depletion regulation of Cl/anion exchanges in AMV were established. The surface cell-specific localization and inhibition of Cl/HCO3 exchange by Na depletion suggests that Cl/HCO3 exchange is involved in vectorial Cl movement, while Cl/OH exchange may be responsible for cell volume and/or cell pH homeostasis in rat distal colon.

**Materials and Methods**

Surface and crypt cell isolation. Surface and crypt cells were isolated from the distal colon of normal and Na-depleted rats (200- to 250-g Sprague-Dawley rats) by divalent chelation techniques, as described previously (16). Normal animals were given Purina rat chow, while Na-free animals were fed an Na-free diet (20 g/day) for 6–7 days, as described previously, which results in elevated serum aldosterone levels (10, 12).1 In brief, everted colonic segments were incubated in isolation buffer that contained 112 mM NaCl, 5 mM KCl, 30 mM EDTA, 20 mM HEPES, and 0.5 mM dithiothreitol. Surface-to-crypt sequential fractions were isolated by shaking and incubating (6 times for 10 min) at 37°C. Surface and crypt cells were sedimented by centrifugation (Beckman GS-6KR; GH–3.8 rotor) at 3,000 g for 2 min, respectively. Surface and crypt cells were distinguished based on ouabain-insensitive and -sensitive K-ATPase activities, respectively (19).

AMV preparation. AMV were prepared from surface and crypt cells by the Percoll gradient and differential centrifuga-

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1 In previous studies, dietary Na depletion and aldosterone infusion via minipumps produced identical changes in Na, Cl, and K transport in rat distal colon (22–24). In addition, serum aldosterone levels were similar in both the dietary Na-depleted animals and those infused with aldosterone via minipumps (12). Thus, in the present study, aldosterone is at times used to refer to Na-depleted animals.
tion method of Stieger et al. (21), as described previously (16, 17). Purity of AMV was validated by a 9- to 10-fold enrichment of K-ATPase activity (6). Protein was assayed by the method of Lowry et al. (13).

Uptake studies. Uptake of $^{36}$Cl (NEN, Boston, MA) was performed by the rapid filtration technique, as described previously (15). Both [HCO$_3^-$] and [OH$^-$] gradient-driven $^{36}$Cl uptake were linear for at least up to 15 s. As a result, $^{36}$Cl uptake was characterized at 12 s. Values are means ± SE of triplicate assays; SE <5% are not shown. All experiments were repeated with at least three different membrane preparations.

RESULTS

Previous studies (15) of $^{36}$Cl uptake in AMV prepared from distal colon of normal rats established the presence of both Cl/HCO$_3^-$ and Cl/OH exchanges and provided evidence that these two Cl/anion exchanges are separate and distinct transport processes. Because Na depletes active electroneutral Na-Cl absorption in in vitro ion flux studies performed under voltage-clamp conditions across isolated mucosa of rat distal colon (10, 12), studies of anion-driven $^{36}$Cl uptake were performed in AMV prepared from distal colon of both normal and Na-depleted rats. Figure 1 presents outward [HCO$_3^-$] gradient (Fig. 1A) and outward [OH$^-$] (Fig. 1B) gradient-driven $^{36}$Cl uptake in AMV from distal colon of normal and Na-depleted rats. Similar to our earlier observations (15), both [HCO$_3^-$] gradient and [OH$^-$] gradient stimulated $^{36}$Cl uptake in AMV from normal animals (Fig. 1). In AMV from Na-depleted animals, both [HCO$_3^-$] and [OH$^-$] gradient-driven $^{36}$Cl uptakes were markedly reduced (Fig. 1). Although Na depletion inhibited both Cl/HCO$_3^-$ and Cl/OH exchanges, the inhibition (70%) of Cl/HCO$_3^-$ exchange was significantly greater than that (47%) of Cl/OH exchange. These observations are consistent with the possibility that Na depletion selectively inhibited Cl/HCO$_3^-$ exchange, and, as a consequence, Cl uptake in the Na-depleted animals occurs primarily via Cl/OH exchange.

To provide additional support for the differential inhibition of Cl/HCO$_3^-$ and Cl/OH exchanges by Na depletion, we studied the effect of increasing outward alkaline pH gradient on $^{36}$Cl uptake in AMV from both normal and Na-depleted animals. In this study, intravesicular pH was increased, while extravesicular pH was maintained constant. As shown in Fig. 2A, outward pH changes, the inhibition (70%) of Cl/HCO$_3^-$ exchange was significantly greater than that (47%) of Cl/OH exchange. These observations are consistent with the possibility that Na depletion selectively inhibited Cl/HCO$_3^-$ exchange, and, as a consequence, Cl uptake in the Na-depleted animals occurs primarily via Cl/OH exchange.
Na-depleted animals, the threshold of intravesicular $\mathrm{HCO}_3$ was not stimulated at these pH gradients. In AMV from normal animals, a hyperbolic curve for $^{36}\mathrm{Cl}$ uptake in AMV from normal animals, while in Na-depleted AMV $^{36}\mathrm{Cl}$ uptake was not stimulated at these pH gradients. In AMV from Na-depleted animals, the threshold of intravesicular pH for stimulation of $^{36}\mathrm{Cl}$ uptake was 6.4. Plotting these $^{36}\mathrm{Cl}$ uptakes as a function of $[\mathrm{OH}]$ gradient yielded a sigmoidal curve in AMV from normal animals (indicating the presence of two $\mathrm{Cl}$ transport processes) but a hyperbolic curve for $^{36}\mathrm{Cl}$ uptake in AMV from Na-depleted animals consistent with the presence of a single $\mathrm{Cl}$ transport system (Fig. 2B). These results suggest that one of the Cl/anion exchanges (i.e., $\mathrm{Cl}/\mathrm{HCO}_3$ exchange) in AMV is not expressed (or is functionally inactive) in Na-depleted animals.

Kinetic studies were therefore performed to determine whether $\mathrm{HCO}_3$ gradient-driven $^{36}\mathrm{Cl}$ uptake seen in AMV from Na-depleted animals (Fig. 1A) represents uptake via $\mathrm{Cl}/\mathrm{HCO}_3$ and/or $\mathrm{Cl}/\mathrm{OH}$ exchanges. As shown in Fig. 3, outward $[\mathrm{HCO}_3]$ gradients of 2.5 and 5 mM stimulated $^{36}\mathrm{Cl}$ uptake in AMV from normal animals, but not in AMV from Na-depleted animals. However, $^{36}\mathrm{Cl}$ uptake in AMV from Na-depleted animals was stimulated by $[\mathrm{HCO}_3]$ gradient at 7.5 mM. Lineweaver-Burk plot analyses of these data revealed an apparent $K_m$ for $\mathrm{HCO}_3$ of $6.8 \pm 1.6$ and $28.2 \pm 3.3$ mM for AMV from normal and aldosterone animals, respectively, and maximal velocity ($V_{\text{max}}$) for $\mathrm{Cl}$ of $2.6 \pm 0.6$ and $0.8 \pm 0.2$ nmol·mg protein$^{-1}$·s$^{-1}$ for AMV from normal and aldosterone animals, respectively. Thus Na depletion is associated with a fivefold increase in $K_m$ for $\mathrm{HCO}_3$. The demonstrated Na depletion-mediated decrease in affinity for $\mathrm{HCO}_3$ shown in Fig. 3 is consistent with two alternate possibilities: 1) Na depletion had solely altered $\mathrm{Cl}/\mathrm{HCO}_3$ exchange so that the affinity of $\mathrm{Cl}/\mathrm{HCO}_3$ exchange for $\mathrm{HCO}_3$ was substantially decreased or 2) Na depletion had markedly reduced $\mathrm{Cl}/\mathrm{HCO}_3$ exchange and that, in the absence of $\mathrm{Cl}/\mathrm{HCO}_3$ exchange, $\mathrm{HCO}_3$ was transported via $\mathrm{Cl}/\mathrm{OH}$ exchange, whose affinity for $\mathrm{HCO}_3$ is less than that of $\mathrm{Cl}/\mathrm{HCO}_3$ exchange.

To distinguish between these two possibilities additional kinetic experiments were performed. As shown in Fig. 4, increasing extravesicular $\mathrm{Cl}$ concentration saturated the $[\mathrm{HCO}_3]$ gradient-driven $^{36}\mathrm{Cl}$ uptake in AMV from both normal and Na-depleted animals. Lineweaver-Burk plot analyses of these studies yielded a $K_m$ of $-8.9 \pm 1.1$ mM and a $V_{\text{max}}$ of $2.4 \pm 0.6$ nmol·mg protein$^{-1}$·s$^{-1}$ for $\mathrm{Cl}/[\mathrm{HCO}_3]$ gradient-driven $^{36}\mathrm{Cl}$ uptake for normal animals and a $K_m$ of $24.3 \pm 1.8$ mM and a $V_{\text{max}}$ of $0.8 \pm 0.2$ nmol·mg protein$^{-1}$·s$^{-1}$ for Na-depleted animals. These results demonstrate that the $K_m$ for $\mathrm{Cl}$ for $\mathrm{Cl}/\mathrm{HCO}_3$ exchange is increased almost threefold in Na-depleted animals compared with normal animals. Similarly, DIDS inhibition kinetics were also determined for $\mathrm{Cl}/\mathrm{HCO}_3$ exchange in normal and Na-depleted animals. Figure 5 reveals that Na depletion increased $K_i$ for DIDS of $\mathrm{ClHCO}_3$ by 15-fold ($K_i$ for DIDS of normal vs. Na depletion: 5.9 vs. 91.1 $\mu$M). Both the $K_m$ for $\mathrm{Cl}$ (24.3 $\pm$ 1.8 mM) and the $K_i$ for DIDS (91.1 $\pm$ 3.6 $\mu$M) for $\mathrm{HCO}_3$ gradient-driven $^{36}\mathrm{Cl}$ uptake in AMV of Na-depleted animals are almost identical to those ($K_m$ of 22.6 mM for $\mathrm{Cl}$ and $K_i$ of 106.0 $\mu$M) for $\mathrm{HCO}_3$ gradient-driven uptake in AMV of normal animals.

2 The presence of a sigmoidal relationship between $[\mathrm{OH}]$ and $^{36}\mathrm{Cl}$ uptake in normal animals in Fig. 2B relies heavily on the experimental data points at low [OH]. As a result, the following alternate interpretation of this data is also possible: 1) that hyperbolic relationships are present in both groups, and 2) that Na depletion does not alter $K_m$ for OH but reduces $V_{\text{max}}$ by $-50\%$, which is a reflection of inhibition of $\mathrm{Cl}/\mathrm{HCO}_3$ exchange.

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**Fig. 3.** Effect of $\mathrm{HCO}_3$ concentration ([HCO$_3$]) gradients. AMV from normal (○) and Na-depleted (●) rat colon were preloaded with 50 mM HEPES-Tris (pH 7.5), 5 mM NMG gluconate, and varying concentrations of KHCO$_3$ (0 or 4.5 mM 0.5% CO$_2$; 7.5 mM 1% CO$_2$; 13.6 mM 1.5% CO$_2$; 31.8 mM 3.5% CO$_2$; 45.4 mM 5% CO$_2$; 68.1 mM 7.5% CO$_2$; 90.8 mM 10% CO$_2$; 154 mM 17% CO$_2$) and potassium gluconate in medium containing 150 mM potassium gluconate, 5 mM $^{36}$Cl-NMG, and 50 mM HEPES-Tris (pH 7.5). Vesicular $[\mathrm{HCO}_3]$ were maintained by gassing with appropriate CO$_2$ mixture for 30 min. Uptake was measured under appropriate gas atmosphere. Uptake was also measured in presence of 1 mM DIDS. Absolute values presented were calculated by subtracting uptake obtained in presence of DIDS from that in its absence. All media contained 25 $\mu$M valinomycin and 0.8% ethanol.

**Fig. 4.** Effect of $\mathrm{Cl}$ concentrations ([Cl]) on $\mathrm{HCO}_3$ gradient-driven $^{36}\mathrm{Cl}$ uptake on AMV from normal (○) and Na-depleted (●) distal colon were preloaded with 150 mM KHCO$_3$, 5 mM NMG gluconate, and 50 mM HEPES-Tris (pH 7.5). Uptake was measured for 12 s by incubating AMV in incubation medium containing 50 mM HEPES-Tris (pH 7.5), 36Cl-NMG, and varying concentrations of KCl. Isosmolarity was maintained by varying potassium gluconate concentrations. Uptake was also measured in presence of 1 mM DIDS. Absolute values presented were calculated by subtracting uptake obtained in presence of DIDS from that in its absence. Vesicles were gassed for 30 min, and uptake were performed under 17% CO$_2$ atmosphere. All media contained 25 $\mu$M valinomycin and 0.8% ethanol.
µM for DIDS (15)] previously established for Cl/OH exchange in normal animals (15).

These results suggest that HCO₃ stimulates ³⁶Cl uptake in AMV from Na-depleted animals via Cl/OH exchange, which has a relatively low affinity for HCO₃ and is consistent with the concept that these two Cl/anion exchanges are not only distinct and separate transport processes but are also differentially regulated by Na depletion. The demonstration that Na depletion inhibits Cl/HCO₃ exchange (Fig. 1A) and Na-dependent Cl absorption (10, 12), but not Cl/OH exchange (Fig. 1B), supports the prior speculation that Cl/HCO₃ exchange is responsible for transepithelial Cl movement, while Cl/OH exchange is linked to one or more Cl-dependent epithelial cell functions (i.e., intracellular pH regulation and cell volume regulation) (15).

Because absorptive processes are primarily present in surface cells, the spatial distribution of Cl/HCO₃ and Cl/OH exchanges in surface and crypt cells was also examined. Figure 6A presents the results of outward [HCO₃] gradient-driven ³⁶Cl uptake in AMV from surface and crypt cells. Compared with Cl/HCO₃ exchange in AMV from surface cells, there was a significant reduction in outward [HCO₃] gradient-driven ³⁶Cl uptake in AMV prepared from crypt cells. DIDS-sensitive outward [HCO₃] gradient-driven ³⁶Cl uptake in crypt AMV was 2.4% of that in surface AMV. In contrast, an outward [OH] gradient-driven ³⁶Cl uptake in AMV prepared from crypt cells was 50% of that in AMV prepared from surface cells (Fig. 6B). Thus the distribution of these two apical membrane anion exchanges in surface and crypt cells differ: Cl/HCO₃ exchange is primarily present only in surface cells, while Cl/OH exchange is localized to apical membranes of both surface and crypt cells. These observations provide additional support for the previous speculation (15) that Cl/HCO₃ and Cl/OH exchanges are separate and distinct transport processes.

**DISCUSSION**

The mechanism(s) for the absorption of Na and Cl in the mammalian colon has been well studied in several species, including human tissue, during the past two or more decades (4, 7, 8, 11, 12). The physiology of Cl absorption has been extensively investigated in the rat distal colon by several different complementary methods that have included in vivo luminal perfusion, in vitro ion fluxes across isolated mucosa under voltage-clamp conditions, and uptake measurements by colonic crypt AMV (4, 11). Studies (4, 7, 8, 12) performed across isolated colonic mucosa under voltage-clamp conditions have established the presence of active Cl absorption that is electroneutral, both Na and HCO₃ dependent, and is inhibited by both aldosterone and increases in mucosal cAMP content.

**Fig. 5.** Effect of DIDS on HCO₃ gradient-driven ³⁶Cl uptake. AMV from normal (○) and Na-depleted (●) distal colon were preloaded with 150 mM KHCO₃, 5 mM NMG gluconate, and 50 mM HEPES-Tris (pH 7.5). Uptake was measured for 12 s by incubating AMV in medium containing 150 mM potassium gluconate, 5 mM ³⁶Cl-NMG, 50 mM HEPES-Tris (pH 7.5), and varying concentrations of DIDS. AMV were gassed for 30 min, and uptake was performed under 17% CO₂. Absolute values were calculated by subtracting uptake obtained in the absence of HCO₃ gradients. All media contained 25 µM valinomycin and 0.8% ethanol.

**Fig. 6.** Distribution of Cl/HCO₃ (A) and Cl/OH (B) exchanges along surface-to-crypt cell axis. AMV prepared from surface and crypt cells of normal rat distal colon were preloaded with 50 mM HEPES-Tris (pH 7.5), 5 mM NMG gluconate, and 150 mM of either KHCO₃ (A) or potassium gluconate (B). Uptake was measured for 12 s by incubating AMV in medium containing 150 mM potassium gluconate, 5 mM ³⁶Cl-NMG, 25 µM valinomycin, and 50 mM of either HEPES-Tris (pH 7.5) (A) or MES-Tris (pH 5.5) (B). AMV with HCO₃ were gassed for 30 min, and uptake was measured under 17% CO₂ atmosphere. Uptake was also measured in presence of 1 mM DIDS. Absolute values presented represent DIDS-sensitive uptake that was derived by subtracting uptake in presence of DIDS from that in the absence of DIDS.
Prior studies of Cl/anion exchange in AMV from normal animals indicated the presence of distinct and separate Cl/HCO₃ and Cl/OH exchanges (15). The present results provide additional and compelling evidence that two distinct apical membrane Cl/anion exchanges are present in the rat distal colon and are differentially regulated by Na depletion. First, Cl/HCO₃ exchange is present in AMV from surface cells but not in AMV from crypt cells (Fig. 6). Second, Cl/HCO₃ exchange was substantially inhibited by Na depletion (Fig. 1A), an observation that parallels our prior demonstration that Na depletion inhibits Na/H exchange (17). Although Cl/OH exchange is also partially inhibited by Na depletion (Fig. 1B), data presented in Figs. 2 and 3 provide evidence that the fraction of Cl/OH exchange that appears inhibited by Na depletion represents [OH⁻] gradient-driven ³⁶Cl uptake via Cl/HCO₃ exchange (Fig. 1B). As a result, the inhibition of active electroneutral Na-Cl absorption by Na depletion in the rat distal colon is secondary to the parallel inhibition of both Na/H and Cl/HCO₃ exchanges.

The previous studies (15) also concluded that both OH⁻ and HCO₃⁻ ions were transported by Cl/HCO₃ exchange, whereas Cl/OH exchange only transported the OH⁻ ion. However, those experiments did not directly address whether HCO₃⁻ was also transported by Cl/OH exchange. These present studies also established that the affinity of Cl/HCO₃ exchange for HCO₃⁻ was less than that of Cl/OH for OH⁻. The data presented in Figs. 2 and 3 with AMV from Na-depleted animals revealed that at very low [OH⁻] and [HCO₃⁻] ³⁶Cl uptake was absent (within the experimental limitations of these studies), but that at higher [OH⁻] and [HCO₃⁻] a low rate of ³⁶Cl uptake was observed. These results are consistent with the transport of both OH⁻ and HCO₃⁻ ions by Cl/HCO₃ exchange and with inactivation of Cl/HCO₃ exchange by Na depletion (see Figs. 1A and 6). At higher [OH⁻] and [HCO₃⁻] ³⁶Cl uptake is mediated by Cl/OH exchange, which has a lower affinity for these anions than Cl/HCO₃ exchange. Thus ³⁶Cl uptake represents both Cl/OH and Cl/HCO₃ exchanges in normal animals, while in Na-depleted rats ³⁶Cl uptake is a result only of Cl/OH exchange that has an affinity for both OH⁻ and HCO₃⁻ ions.

Several observations provide the basis for the speculation that Cl/HCO₃ exchange is the mechanism of vectorial, transepithelial Cl absorption. First, absorptive processes are primarily, though not exclusively, located in colonic surface cells, while secretory processes are in crypt cells. Figure 6 demonstrates that Cl/HCO₃ exchange is primarily present in AMV prepared from surface cells and not in AMV from crypt cells. In contrast, Cl/OH exchange is present in AMV from both surface and crypt cells (Fig. 6). Second, in addition to Na depletion's induction of Na channels in apical membrane of the distal colon, Na depletion inhibits both electroneutral Na-Cl absorption and [H⁺] gradient-driven ²²Na uptake (i.e., Na/H exchange) (12, 17). Figure 1A demonstrates that Na depletion substantially inhibits Cl/HCO₃ exchange. Thus these observations indicate that Cl/HCO₃ exchange is present in surface cells and is most likely closely associated with transepithelial Cl movement. Conversely, Cl/OH exchange is present in both surface and crypt cells (Fig. 6) and we propose that Cl/OH exchange is not regulated by Na depletion and not associated with transepithelial Cl movement but rather with the regulation of cell volume and/or intracellular pH.

To date, three different anion exchange genes (AE1, AE2, and AE3) have been cloned from different species (1). However, 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 (1). Although studies in native tissue clearly indicate the presence of anion exchange function in apical membranes (3, 4), expression of AE isoform proteins has not been reported in either small or large intestine. To date, AE2 isoform-specific protein has been expressed in basolateral membranes of rat stomach and intestine and human intestine (2, 6, 20).

In conclusion, the present study establishes the distribution and Na depletion regulation of Cl/anion exchanges in the AMV of rat distal colon. The pattern of surface cells to crypt cells spatial distribution and Na depletion regulation indicate Cl/HCO₃ exchange is responsible for transepithelial Cl movement in apical membranes of rat distal colon. In contrast, Cl/OH exchange that is present in AMV of both surface and crypt cells and not regulated by Na depletion may be responsible for cell volume and/or cell pH homeostasis in rat distal colon.

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