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Chloride transport in human proximal colonic apical membrane vesicles

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Abstract

The mechanism(s) of Cl^- transport across the human colonic apical membranes are not well understood. Apical membrane vesicles (AMV) purified from organ donor proximal colonic mucosa and a rapid millipore filtration technique were utilized to study $^{36}Cl^-$ uptake into these vesicles. Outwardly directed OH^- and HCO_3^- gradient stimulated $^{36}Cl^-$ uptake into these vesicles demonstrating a transient accumulation over equilibrium uptake. Voltage clamping the membrane potential of the vesicles or making them inside positive with K^+/valinomycin failed to influence chloride uptake, indicating that the conductive Cl^- uptake pathway is minimal in proximal colonic AMV. Anion exchange inhibitors, DIDS and SITS (1 mM) inhibited OH^- and HCO_3^- stimulated $^{36}Cl^-$ uptake by $\approx 60\%$. Furosemide also demonstrated a small but significant inhibition of chloride uptake. Amiloride, bumetanide and acetazolamide (1 mM) failed to inhibit ^{36}Cl uptake. HCO_3^- and pH gradient stimulated $^{36}Cl^-$ uptake exhibited saturation kinetics with an apparent K_m for chloride of 4.0 ± 0.7 mM and a V_{max} of 17.8 ± 3.9 nmol/mg per min. Bromide, chloride, nitrate and acetate (50 mM each) inhibited 5 mM ^{36}Cl uptake. Inwardly directed gradients of Na^+, K^+, or Na^+ and K^+ did not stimulate $^{36}Cl^-$ uptake into these vesicles, indicating that uptake of Na^+ and Cl^- in human proximal colonic AMV does not involve Na-Cl or Na-K-2Cl cotransport. The above findings indicate that chloride transport in human proximal colonic AMV involves an electroneutral Cl^-–HCO_3^- (OH^-) exchange process. In view of the previous demonstration of a Na^+-H^+ antiporter in these vesicles, dual ion exchange mechanism of Na^+–H^+ and Cl^-–HCO_3^- in apical membrane domain of human colonocytes is postulated to be the primary mechanism for NaCl absorption in the human proximal colon.

Keywords: Chloride–bicarbonate (hydroxide) ion exchange; Chloride transport; Hydroxide ion gradient; Bicarbonate ion gradient; Sodium chloride absorption; (Human colon)

1. Introduction

Previous studies of ion transport in the human colon have employed either in vivo steady state perfusion techniques [1–3] or in vitro preparations of intact mucosa using short-circuit current method [4–6]. These studies indicated that the chloride absorption in the human colon may involve an electroneutral chloride-bicarbonate exchange process that occurred independently of the presence of Na^+ in the bathing solution [2,4,6]. However, detailed studies of this electroneutral process were not conducted and the mechanism of coupling with Na^+ was not investigated. The exact mechanism(s) of Cl^- absorption in the human colon at the apical membrane level, i.e., involving coupled NaCl absorption by NaCl or Na-K-2Cl cotransport or dual ion exchanges of Na^+-H^+ and Cl^--HCO_3^-(OH^-) has not been examined.

A number of studies using laboratory animals have previously been performed to understand the mechanism(s) of mammalian colonic electrolyte transport [7–11]. Studies in the human intestine from our group [12–15] and others [5,6,16] have clearly indicated that the information gained on the transport characteristics of Na^+ and Cl^- in various animal species can not be simply extrapolated to the human gastrointestinal tract due to significant differences with respect to normal transport characteristics as well as hormonal regulation [2,4–8,16]. Therefore, it is important to examine the electrolyte transport characteristics of the human colon itself.

Until recently, the ion transport studies in the human colon at the plasma membrane level have been lacking,

Abbreviations: AMV, apical membrane vesicles; DIDS, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; MES, 2-(*N*-morpholino)ethanesulfonate; N-MG, *N*-methyl-D-glucamine; N-MGG, *N*-methyl-D-glucamine gluconate; SITS, 4-acetamido-4′-isothiocyano-2,2′-disulfonic acid stilbene; Tris, tris(hydroxymethyl)aminomethane.

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due to inability to isolate the plasma membranes from the colonocytes. Our laboratory has recently developed a technique for the isolation and purification of human colonic apical membrane vesicles from colonic tissues harvested from organ donor colons [17] and utilizing these vesicle preparations have partially characterized the Na^+ transport mechanisms in both the proximal [12] and distal [13] human colon. The present studies were, therefore, undertaken to investigate Cl^- transport mechanisms in the apical membranes of proximal human colon. Our studies demonstrate that (i) an electroneutral chloride-bicarbonate exchange is the principal mode of chloride absorption in the proximal human colon; (ii) conductive chloride uptake is minimal. Based on these findings and the previous demonstration of a sodium-proton antiporter in these apical membranes by our group [12], a model for NaCl transport across the apical membranes of the proximal human colon has been proposed.

2. Methods

2.1. Materials

Valinomycin, 4-acetamido-4′-isothiocyano-2,2′-disulfonic acid stilbene (SITS), 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS), amiloride, bumetanide and acetazolamide were obtained from Sigma (St. Louis, MO). Stock solutions (1 M) of *N*-methyl-D-glucamine gluconate (N-MGG) were made by titrating 1 M *N*-methyl-D-glucamine (N-MG) with solid D-gluconic acid lactone. All other materials were obtained from either Fisher Scientific (Fairlawn, NJ) or Sigma, unless otherwise stated and were of the highest purity available.

2.2. Isolation of human proximal colonic apical membrane vesicles

These investigations were approved by the Institutional Review Board of the University of Illinois at Chicago. Colons from 8 healthy adult organ donors were obtained after harvest of transplantation organs. The cecum was discarded and the remaining large intestine was divided into two equal parts, proximal and distal. After cleaning the contents, the mucosa of proximal colon was scraped and frozen at −80°C. Purified apical membranes were prepared from thawed mucosa utilizing divalent cation (Mg^{2+}) chelation and differential centrifugation technique [17]. All the steps were carried out on ice to minimize any cellular activity or metabolism. The purity of membrane vesicles and the degree of contamination with intracellular organelles were assessed by appropriate marker enzymes. Membrane vesicles demonstrated approx. 8–11-fold enrichment in cysteine sensitive alkaline phosphatase activity (colonic apical membrane marker) compared to crude homogenate. The corresponding values for succinate dehydrogenase, NADPH–cytochrome-*c* reductase, and sodium-potassium dependent adenosine triphosphatase, marker enzymes for mitochondrial, microsomal, and basolateral membranes, respectively, ranged from 0.5 to 2.2 in all membrane preparations [12]. The sidedness of these vesicles was determined by estimating the activity of the marker enzyme cysteine-sensitive alkaline phosphatase in vesicles opened up by 0.05% Triton X-100. These vesicles were found to be ≈ 80–85% right side out. For loading vesicles with various constituents, the desired intravesicular medium buffer was utilized in the last two centrifugation and in all the resuspension steps for the purification procedure and have been described in the figure legends.

After the final suspension, the vesicles were used for uptake studies either within 1–2 h of purification or were quick frozen and stored at −80°C for use within 2 weeks of preparation without any significant loss of transport activity. The membrane protein was assessed by Bradford technique, using bovine plasma globulin as standard [18].

2.3. ^{36}Cl uptake studies

The apical membrane vesicles (20 μl) were incubated in incubation media (80 μl) with known buffer composition (described in detail in the figure legends or table legends of Section 2), and containing 5 mM radioactive $^{36}Cl^-$ as previously described by our laboratory [19]. The transport was studied at 25°C in a water bath with uniform temperature. The transport was stopped at various time points using 3 ml of ice-cold stop solution containing 50 mM Tris/Mes, 100 mM N-MGG, 150 mM mannitol and 0.285 mM $KHCO_3$, pH 5.5. The diluted sample was rapidly filtered utilizing a rapid filtration technique, employing 0.65 μm nitrocellulose filters. Filters were further washed twice with 3 ml ice-cold stop solution. The filters were then dissolved in Filtercount and the radioactivity measured in a Packard TR1600 liquid scintillation counter (Packard Inc., Downers Grove, IL). All values were corrected for nonspecific $^{36}Cl^-$ binding to filters and/or vesicles by subtracting radioactivity present in zero time vesicle blanks. In studies in which the effect of valinomycin on OH^- gradient or OH^- and HCO_3^- gradient-stimulated $^{36}Cl^-$ uptake was evaluated, the valinomycin (20 μM final concentration) was added from ethanolic stock solutions resulting in a final ethanol concentration of 0.5% (v/v) of the incubation media during all measurements. All the HCO_3^- containing media were gassed with 5% CO_2/95% N_2.

2.4. Statistical analysis

All experiments were performed using at least three or four freshly isolated membrane preparations prepared from proximal colons of different organ donors. Results are expressed as mean ± S.E. Paired or unpaired Student's *t*-tests were used in statistical analysis as appropriate. A P value of < 0.05 was considered statistically significant.

3. Results

3.1. Time course of HCO_3^-/OH^- gradient stimulated ^{36}Cl uptake

Initial studies were designed to demonstrate the effect of an outwardly directed OH^- gradient (pH 7.8 in/5.5 out) as well as an outwardly directed bicarbonate gradient on the time course of ^{36}Cl (5 mM) uptake. The results, as shown in Fig. 1, demonstrate that both the outwardly directed OH^- as well as HCO_3^- gradient stimulated $^{36}Cl^-$ uptake into the apical membrane vesicles. The peak uptake of chloride in presence of hydroxyl and bicarbonate gradient was significantly higher ($P < 0.05$) than the uptake of chloride at equilibrium (120 min) or in the absence of a pH or bicarbonate gradient. Thus chloride uptake demonstrated transient accumulation of ^{36}Cl or an 'overshoot' phenomenon, indicative of a carrier mediated transport process for chloride in these vesicles.

3.2. Effect of transmembrane potential gradient on ^{36}Cl uptake

To determine the existence of conductive pathways for chloride transport in the human proximal colonic apical membrane vesicles, the effect of K^+/valinomycin induced

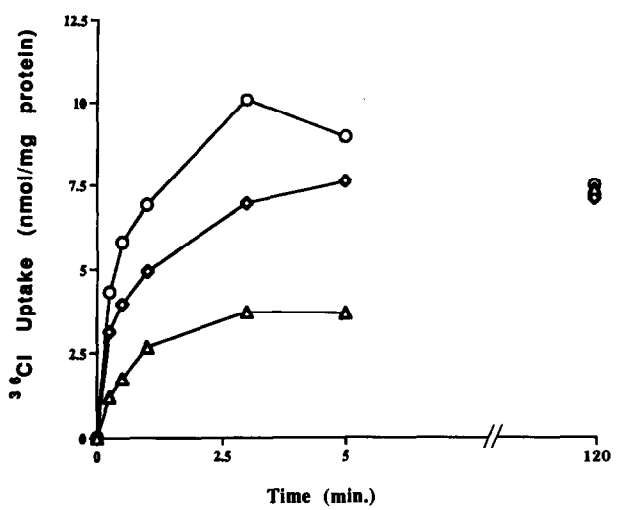

Fig. 1. Time course of HCO_3^-/OH^- gradient stimulated ^{36}Cl uptake. ^{36}Cl (5 mM) uptake was determined at 25°C by diluting membrane vesicles (≈ 80–100 μg protein) preloaded with either: (○) 50 mM Tris/Hepes, 50 mM $KHCO_3$, 50 mM *N*-methylglucamine gluconate (N-MGG),150 mM mannitol, pH 7.5; (◇) 50 mM Tris/Hepes, 100 mM N-MGG, 150 mM mannitol, pH 7.5, or (△) 50 mM Tris/Mes, 100 mM N-MGG, 150 mM mannitol, pH 5.5; into a reaction medium containing 50 mM Tris/Mes, 100 mM N-MGG, 150 mM mannitol, pH 5.5 (◇ and △) or 50 mM Tris/Mes, 100 mM N-MGG, 150 mM mannitol and 0.285 mM $KHCO_3$, pH 5.5 (○). All bicarbonate containing buffers were gassed with 5% CO_2 /95% N_2. Values are representative of 5–7 separate preparations. These results demonstrate that outwardly directed OH^- and HCO_3^- gradients stimulated $^{36}Cl^-$ uptake into the apical membrane vesicles (AMV), exhibiting a transient accumulation over equilibrium uptake.

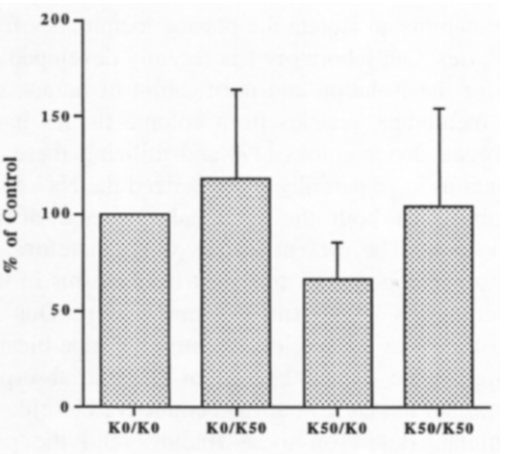

Fig. 2. Effect of transmembrane potential gradient on ^{36}Cl uptake. Membrane vesicles were loaded with one of the two buffers: 50 mM Tris/Hepes, 100 mM N-MGG, 150 mM mannitol, pH 7.5 (K0), or, 50 mM Tris/Hepes, 50 mM N-MGG, 50 mM K^+ gluconate, 150 mM mannitol, pH 7.5 (K50). The incubation media used were either K0 or K50 along with valinomycin (20 μM final concentration). K0/K0 represents K0 in = K0 out, K0/K50 represents K0 buffer within the vesicles and K50 outside, K50/K0 represents K50 buffer within the vesicles and K0 outside, and, K50/K50 represents K50 in = K50 out. Voltage clamping the membrane potential of the vesicles or making them inside positive with K^+/valinomycin failed to influence chloride uptake ($P > 0.05$) compared to controls, indicating that the conductive Cl^- uptake pathway is minimal in proximal colonic AMV. Values are represented as % of control and are mean ± S.E. of three or four separate membrane preparations, control values (K0/K0) were 3.20 ± 1.14 nmol/mg protein/15 s.

voltage potential on chloride uptake was determined. The effects of applying an intravesicular negative (K^+in > K^+out + valinomycin) or positive (K^+in < K^+out + valinomycin) membrane potential on Cl^- uptake in the absence of a transvesicular OH^- and HCO_3^- gradient (pH 7.5 in/7.5 out) were assessed. These results were compared with vesicles in the absence of a transvesicular potential, i.e., voltage clamping (K^+in = K^+out + valinomycin). As shown in Fig. 2, voltage clamping of the vesicles (K^+in = K^+out) or making them inside positive with K^+/valinomycin failed to significantly influence chloride uptake, indicating that the conductive Cl^- uptake pathway is minimal in proximal colonic AMV.

3.3. Effect of transport inhibitors on ^{36}Cl uptake

To further characterize this transport process, the effect of a number of carrier-mediated transport inhibitors (1 mM each) on pH and HCO_3^- stimulated $^{36}Cl^-$uptake (15 s) into the vesicles was examined. As shown in Table 1, DIDS and SITS, well known anion exchange inhibitors [20] significantly ($P < 0.05$) inhibited HCO_3^- and OH^- gradient stimulated $^{36}Cl^-$ uptake by ≈ 60%. Amiloride (a Na^+-H^+ exchange inhibitor), bumetanide (Na-Cl and Na-K-2Cl cotransport inhibitor), and acetazolamide (carbonic

Table 1
Effect of transport inhibitors on pH and bicarbonate gradient stimulated chloride uptake [a]

Inhibitors (1 mM)	% of control
DIDS	42.3 ± 10.7 *
SITS	39.2 ± 12.2 *
Amiloride	108.7 ± 26.5
Bumetanide	75.0 ± 25.4
Furosemide	73.4 ± 12.7 *
Acetazolamide	81.0 ± 11.8

[a] Expressed as % of control with S.E. (* $P < 0.05$).
Membrane vesicles preloaded with 50 mM Tris/Hepes, 50 mM $KHCO_3$, 50 mM N-MGG, 150 mM mannitol (pH 7.5), were diluted into incubation medium consisting of 50 mM Tris/Mes, 100 mM N-MGG, 150 mM mannitol, 0.285 mM $KHCO_3$ (pH 5.5). Cl^- uptake at 30 s was determined in the presence and absence of 1 mM concentrations of each inhibitor. Values represent mean ± S.E. of four or five separate preparations. Anion exchange inhibitors like DIDS and SITS (1 mM) inhibited HCO_3^- and OH^- stimulated $^{36}Cl^-$ uptake by ≈ 60%. Amiloride, bumetanide, and acetazolamide (1 mM) failed to inhibit $^{36}Cl^-$ uptake. Furosemide also caused a small but significant inhibition. Values represent mean ± S.E. of three or four separate membrane preparations.

anhydrase inhibitor) failed to significantly inhibit $^{36}Cl^-$ uptake. Furosemide, however, showed a small but significant inhibition of chloride transport. Taken together, these observations support the presence of a carrier mediated chloride-bicarbonate exchange process in human proximal colonic apical membrane vesicles.

3.4. Effect of anions on ^{36}Cl uptake

The effect of various anions on Cl^- uptake was investigated to determine anion specificity of the exchanger. As shown in Fig. 3, 50 mM cis concentrations of chloride, bromide, acetate and nitrate significantly inhibited ($P <$ 0.05) 5 mM $^{36}Cl^-$ uptake into these vesicles. These findings suggest that acetate, nitrate and bromide could also serve as alternate substrates for this antiporter.

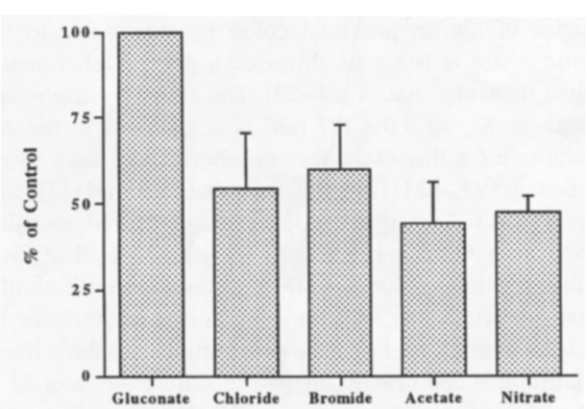

Fig. 3. Effect of anions on ^{36}Cl uptake. Vesicles preloaded with 50 mM Tris/Hepes, 50 mM $KHCO_3$, 50 mM N-MGG, 150 mM mannitol (pH 7.5) were incubated in buffer containing 50 mM Tris/Mes, 100 mM N-MGG, 150 mM mannitol (pH 5.5). The $^{36}Cl^-$ uptake was studied in the presence of 50 mM concentrations of various anions. $^{36}Cl^-$ uptake in the presence of gluconate was considered as 100%. Values are presented as percent of control and represent means ± S.E. of three separate preparations. Bromide, chloride, nitrate, acetate (50 mM each) significantly inhibited 5 mM $^{36}Cl^-$ uptake ($P < 0.05$ or less). Control values for gluconate were 5.69 ± 0.83 nmol/mg protein/15 s.

3.5. Kinetics of Cl^--HCO_3^- exchange

The effect of increasing concentrations of Cl^- on pH and HCO_3^- stimulated $^{36}Cl^-$ uptake was investigated to determine the kinetic characteristics of the transport process. ^{36}Cl uptake at 15 s ($^{36}Cl^-$ uptake was shown to be linear for at least 15 s even at 25 mM Cl^- concentrations) was determined using increasing concentrations (2–25 mM) of $^{36}Cl^-$. As shown in Fig. 4, hydroxyl and bicarbonate gradient dependent chloride uptake demonstrated saturability in the presence of increasing concentrations of Cl^-. The actual uptake values were corrected by subtracting the uptake in the absence of a pH and bicarbonate gradient. Lineweaver-Burk plot demonstrated a straight line with an apparent K_m for Cl^- of 4.0 ± 0.7 mM and a V_{max} of 17.8 ± 3.9 nmol/mg/min. These results are again consistent with a carrier mediated transport process for chloride.

3.6. Effect of inwardly directed Na^+, K^+ or $Na^+ + K^+$ on $^{36}Cl^-$ uptake

Electroneutral NaCl uptake across apical membranes of human proximal colon can be explained by either a (i)

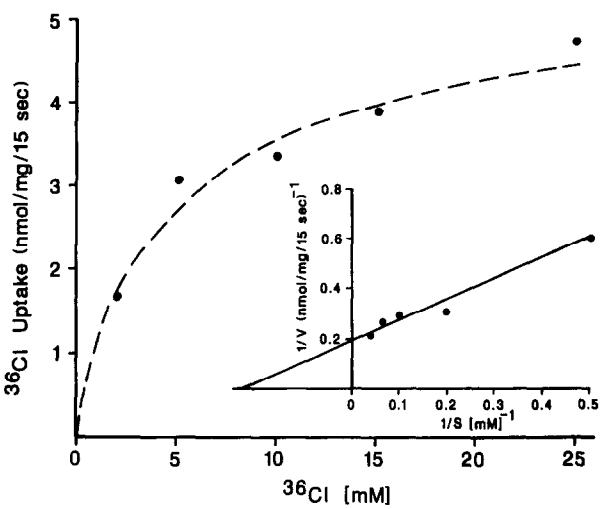

Fig. 4. Kinetics of Cl^--HCO_3^- exchange. HCO_3^- and OH^- gradient (pH 7.5 in/pH 5.5 out) stimulated $^{36}Cl^-$ uptake was determined at increasing extravesicular concentrations of Cl^- (2–25 mM). Vesicles preloaded with 50 mM Tris/Hepes, 50 mM $KHCO_3$, 50 mM N-MGG, 150 mM mannitol, (pH 7.5) were diluted into the incubation medium containing 50 mM Tris/Mes, 100 mM N-MGG, 150 mM mannitol, 0.285 mM $KHCO_3$ (pH 5.5) and 2–25 mM $^{36}Cl^-$ (final concentrations). Total salt concentration was kept constant on both sides of vesicles by altering N-MGG concentration. ^{36}Cl uptake at 15 s were determined and results shown are representative of four separate membrane preparations. The actual uptake values were corrected by subtracting the uptake in the absence of a pH and bicarbonate gradient. Lineweaver-Burk plot demonstrated a straight line with an apparent K_m for Cl^- of 4.0 ± 0.7 mM and a V_{max} of 17.8 ± 3.9 nmol/mg/min. Values represent mean ± S.E. of three or four separate membrane preparations.

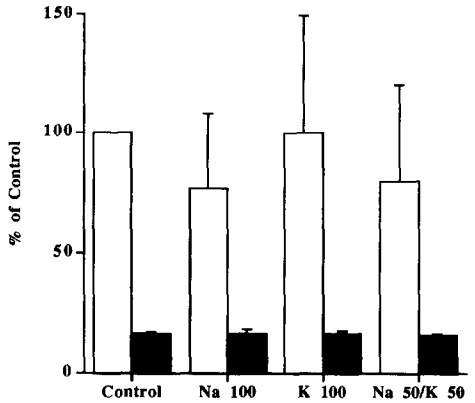

Fig. 5. Effect of inwardly directed Na^+, K^+ or $Na^+ + K^+$ gradients on $^{36}Cl^-$ uptake in presence (open bars) or absence of a HCO_3^- and OH^- gradient (closed bars). Vesicles were preloaded with 50 mM Tris/Hepes, 50 mM $KHCO_3$, 50 mM N-MGG, 150 mM mannitol (pH 7.5) and diluted in incubation media containing 50 mM Tris/Mes, 150 mM mannitol, 0.285 mM $KHCO_3$ (pH 5.5), with either 100 mM N-MGG, 100 mM Na^+ gluconate, 100 mM K^+ gluconate or 50 mM Na^+ gluconate + 50 mM K^+ gluconate (open bars). For studies in the absence of a HCO_3^- and OH^- gradient, vesicles were preloaded with 50 mM Tris/Hepes, 100 mM N-MGG, 150 mM mannitol (pH 7.5) and diluted in incubation media containing 50 mM Tris/Hepes, 150 mM mannitol (pH 7.5), with either 100 mM N-MGG, 100 mM Na^+ gluconate, 100 mM K^+ gluconate or 50 mM Na^+ gluconate + 50 mM K^+ gluconate (closed bars). The $^{36}Cl^-$ uptake at 15 s in the absence of inwardly directed Na^+ and K^+ (controls) is taken as 100%. All the other values are expressed as % of control. Inwardly directed gradients of Na^+, K^+ or $Na^+ + K^+$ did not stimulate $^{36}Cl^-$ uptake into these vesicles compared to controls both in the presence as well as the absence of a HCO_3^- and OH^- gradient ($P > 0.05$), indicating that coupling of Na^+ and Cl^- in human proximal colonic AMV does not involve Na-Cl or Na-K-2Cl cotransport. Values represent mean ± S.E. of three or four separate membrane preparations. Control values for Cl^- uptake in the presence of an outwardly directed HCO_3^- and OH^- gradient were 3.26 ± 0.69 nmol/mg protein/15 s and in the absence of gradients were 0.71 ± 0.03 nmol/mg protein/15 s.

cotransport of Na-Cl or Na-K-2Cl by a single transporter, or (ii) dual ion exchange of Na^+-H^+ and Cl^--$HCO_3^-(OH^-)$. To address this issue, experiments were carried out to examine the presence of a possible Na-Cl or Na-K-2Cl cotransport process in these vesicles. The effect of inwardly directed Na^+ and K^+ gradient on $^{36}Cl^-$ uptake was evaluated in the presence as well as absence of an outwardly directed OH^- and HCO_3^- gradient. As shown in Fig. 5, inwardly directed gradients of Na^+ (100 mM), K^+ (100 mM) or $Na^+ + K^+$ (50 mM each) failed to significantly influence ($P > 0.05$) $^{36}Cl^-$ uptake into these vesicles under both the conditions, i.e., with or without an outwardly directed OH^- and HCO_3^- gradient, indicating that coupling of Na^+ and Cl^- in human proximal colonic AMV does not involve Na-Cl or Na-K-2Cl cotransport process.

4. Discussion

Chloride bicarbonate exchangers are widely distributed plasma membrane transport proteins implicated in maintenance of intracellular pH, volume and transcellular transport of Cl^- and HCO_3^-. Our present studies have, for the first time, demonstrated and partially characterized the presence of an electroneutral Cl^--$HCO_3^-(OH^-)$ exchanger in the human proximal colonic AMV. The findings consistent with the presence of an electroneutral Cl^--$HCO_3^-(OH^-)$ exchanger are: (i) outwardly directed OH^- and HCO_3^- gradients stimulated $^{36}Cl^-$ uptake into the AMV demonstrating a transient accumulation over equilibrium uptake; (ii) $^{36}Cl^-$ uptake was potential insensitive; (iii) $^{36}Cl^-$ uptake exhibited saturation kinetics; (iv) anion exchange inhibitors like DIDS and SITS (1 mM) inhibited HCO_3^- stimulated $^{36}Cl^-$ uptake; (v) cis presence of anions such as bromide, chloride, nitrate and acetate (50 mM each) inhibited 5 mM $^{36}Cl^-$ uptake, indicating a possible competition for carrier by alternative substrates; (vi) inwardly directed gradients of Na^+, K^+ or $Na^+ + K^+$ did not stimulate $^{36}Cl^-$ uptake into the vesicles, indicating that coupling of Na^+ and Cl^- in human proximal colonic AMV does not involve Na-Cl or Na-K-2Cl cotransport.

Our findings suggest that an electroneutral Cl^--$HCO_3^-(OH^-)$ exchange appears to be the principal mode of Cl^- transport across the apical membranes of the human proximal colon. Conductive pathways for chloride appear to play minimal role, if any, in chloride absorption in this segment of the colon. This finding is somewhat compatible with the observations of Sellin and DeSoigne [6], who observed lower conductance in general in proximal segments of colon compared to more distal segments. He suggested that proximal colon may be an electrically 'tighter' epithelium than other regions of the colon.

The human colonic $Cl^-/HCO_3^-(OH^-)$ antiporter exhibits characteristics similar to Cl^--HCO_3^- antiporters of other transporting epithelia. For example, the kinetics of the chloride-bicarbonate exchanger in apical membrane vesicles of human proximal colon is comparable to the kinetic characteristics of this exchanger in other mammalian intestinal tissues [21–23]. The chloride-bicarbonate exchanger K_m of 4.0 ± 0.7 mM is comparable to the K_m of chloride for this exchanger in other tissues such as rat duodenum (9.8 mM) [21], rabbit ileum (13.3 mM) [22] and human ileum (3.5 mM) [23]. Also, compared to other transporting epithelia [21,22], the Cl^--HCO_3^- exchanger in human proximal colonic AMV appears to exhibit similar anion specificity, as well as inhibition characteristics by transport inhibitors. For example, similar to rabbit ileum [22], bromide and nitrate can also substitute for chloride in this exchange mechanism, in the Cl^--HCO_3^- exchanger of human proximal colonic apical membrane vesicles.

DIDS and SITS (1 mM each) produced 60% inhibition, in OH^- and HCO_3^- gradient driven $^{36}Cl^-$ uptake. These findings indicate that although these inhibitors are specific for anion exchange processes, human proximal colonic $Cl^-/HCO_3^-(OH^-)$ antiporter appears to be relatively less sensitive to inhibition by DIDS and SITS similar to a number of other intestinal anion exchangers, e.g., of rabbit

ileum [22] and human ileum [23–25]. The specificity of these agents in inhibiting human proximal colonic apical membrane $Cl^-/HCO_3^-(OH^-)$ antiporter is further strengthened as 1 mM concentrations of both the DIDS and SITS had no significant effect on Na^+-H^+ exchange activities in apical membranes of the human proximal [12] and distal colon [13]. Another possible explanation for poor inhibition by Stilbene derivatives may also be a result of possible competition with the substrate [20]. Further studies to evaluate the mechanism of inhibition by stilbene derivatives will be required to confirm this assumption. Furosemide is frequently used as an inhibitor for Na-Cl or Na-K-2Cl cotransport activities [26,27], however, its ability to inhibit anion exchange processes have also been documented in brush border membrane vesicle preparations of rat and rabbit small intestine [22,28], as well as in erythrocytes [29]. Similar to the above findings, furosemide (1 mM) also produced a small but significant inhibition in OH^- and HCO_3^- stimulated Cl^- uptake. Bumetanide (1 mM) also exhibited a small but non-significant ($P > 0.05$) inhibition of hydroxyl and bicarbonate stimulated Cl^- uptake into these vesicles. The small inhibition of chloride uptake by bumetanide and furosemide may also be due to minor cross-contamination of apical membranes by basolateral membranes.

Recent studies by Rajendran and Binder [30] have suggested that in rat colonic apical membrane vesicles the mechanism of chloride uptake involved two distinct transporters including a Cl^--OH^- and Cl^--HCO_3^- transporters. Unlike our current studies, in their studies although HCO_3^- gradient-stimulated Cl^- uptake was further stimulated by the presence of enhanced pH gradient [30], but the pH gradient-stimulated ^{36}Cl uptake was not further enhanced by a HCO_3^- gradient. These differences in mechanisms of chloride uptake in rat versus human colonic apical membranes further emphasize the importance of investigations directly performed with human tissues to better understand the pathophysiologic basis of human colonic diseases.

4.1. Proposed model for apical NaCl absorption in the human proximal colon

Previous in vitro studies utilizing short circuit current techniques [6], have suggested that NaCl absorption in proximal human colon may predominantly be represented by an electroneutral process. However, to date, the mechanism involved, i.e., dual ion exchange of Na^+-H^+ and Cl^--HCO_3^- or Na-Cl or Na-K-2Cl had not been investigated. Utilizing AMV, previous studies from our laboratory [12] have demonstrated the presence of a Na^+-H^+ exchange process in the proximal human colon. The present studies also directly demonstrate the existence of an anion exchanger on this membrane. In light of our previous observations, a model of electroneutral sodium chloride absorption in the proximal human colon can now be postulated (Fig. 6). As shown in Fig. 6, NaCl uptake at the

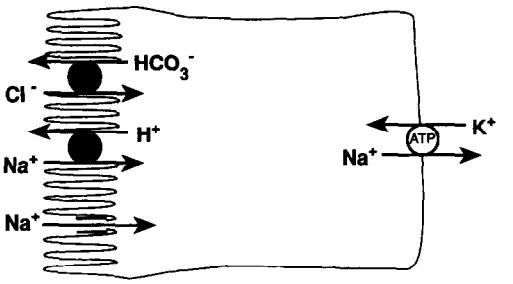

Fig. 6. Postulated model of electroneutral sodium chloride absorption in the proximal human colon.

apical membrane domain of epithelial cells of the human proximal colon is postulated to involve dual ion exchanges of Na^+-H^+ and Cl^--HCO_3^-. This hypothesis is further supported by our data demonstrating the lack of direct coupling of chloride movement to Na^+ or Na^+ and K^+ via a Na^+-Cl^- or Na^+-K^+-$2Cl^-$ cotransport, as the inwardly directed sodium or potassium gradient did not stimulate chloride uptake into these vesicles.

The intracellular pH might serve as the indirect coupling force between these two parallel ion exchange processes. As previously reported, sodium can enter the cell across the apical membrane by an electroneutral Na^+-H^+ exchange mechanism [12]. The movement of sodium into the colonocyte in the direction of a downhill Na^+ concentration can provide the driving force for the uphill efflux of protons from the cell. The proton efflux could lead to a decreased concentration of H^+ inside the cell and can result in an intracellular base surplus. This could result in an outwardly directed HCO_3^- and OH^- gradient, which can drive the chloride uptake into the cell by the anion exchanger, i.e., Cl^--$HCO_3^-(OH^-)$ exchanger. Therefore, an electroneutral NaCl uptake into the colonocyte could possibly be a result of parallel operation of both the Na^+-H^+ and Cl^--$HCO_3^-(OH^-)$ exchangers. Further studies are required to vigorously test this model of NaCl absorption in the human colon.

This postulated dual ion exchange model of NaCl uptake in the proximal human colon is comparable to the role of anion exchanger activities in the apical membrane of several absorptive epithelia, including rat distal colon [19], mammalian small intestine [22,31] and Necturus proximal tubule [32]. However, in the intestine of the flounder [33] and the ascending loop of Henle of the mammalian kidney [34] electroneutral sodium chloride absorption has been attributed to a Na^+-K^+-Cl^- cotransport mechanism in the apical membrane, rather than the dual ion exchange model identified in the mammalian intestine [22,31].

Recent studies have established the presence of an anion exchanger (AE) gene family [35,36], consisting of at least three members. The isoform AE-1 is the band 3 chloride-bicarbonate exchanger expressed in erythrocytes and AE-3 has been isolated from cardiac and neural tissues [35,36]. Studies by Chow et al. [37] have shown the

presence of AE-2 isoform localized in the brush border membrane and not the basolateral membrane of rabbit ileal villus cells. The presence of an AE-3 transcript in rat distal small intestine has also been shown [38]. Although the molecular nature of the chloride-bicarbonate exchanger present in apical membranes of human proximal colon is not known, we are currently involved in identifying this anion exchanger isoform present in the human colon. Identification and characterization of the molecular isoform of this exchanger will be of great importance for understanding the molecular mechanisms of regulation of NaCl absorption in the human colon.

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