J. Membrane Biol. 141, 51–58 (1994)

The Journal of
Membrane
Biology

Apical Membrane Cl-Butyrate Exchange: Mechanism of Short Chain Fatty Acid Stimulation of Active Chloride Absorption in Rat Distal Colon

V.M. Rajendran, H.J. Binder

Department of Internal Medicine, Yale University, 333 Cedar Street, New Haven, Connecticut 06510

Received: 3 November 1993/Revised: 9 March 1994

Abstract. The cellular model of short chain fatty acid stimulation of electroneutral Na-Cl absorption in large intestine proposes that SCFA, following its uptake across the apical membrane, recycles and is coupled to functional Na-H and Cl-short chain fatty acid exchanges. To establish the presence of a Cl-butyrate exchange (used as a model short chain fatty acid), studies of ^{36}Cl and ^{14}C-butyrate uptake across apical membrane vesicles of rat distal colon were performed. An outward butyrate-gradient stimulated transient accumulation of ^{36}Cl uptake that was not inhibited by pH clamping with valinomycin (a K ionophore) and FCCP (a proton ionophore). Outward butyrate-gradient-stimulated ^{36}Cl uptake was inhibited by 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) with a half-maximal inhibitory concentration (IC_{50}) of 68.4 μM, and was saturated by both increasing extravesicular Cl concentration (K_m for Cl of 26.8 ± 3.4 mM and a V_{max} of 12.4 ± 0.6 nmol/mg protein · 9 sec) and increasing intravesicular butyrate concentration (K_m for butyrate of 5.9 mM and a V_{max} for Cl of 5.9 nmol/mg protein · 9 sec). ^{36}Cl uptake was also stimulated by outward gradients of other short chain fatty acids (e.g., propionate, acetate and formate). In contrast, an outward Cl gradient failed to enhance ^{14}C-butyrate uptake. Extravesicular Cl more than extravesicular butyrate enhanced ^{36}Cl efflux from apical membrane vesicles. These studies provide compelling evidence for the presence of an electroneutral, pH-activated, Cl-butyrate exchange which in concert with Na-H exchange is the mechanism by which butyrate stimulates electroneutral Na-Cl absorption.

Key words: Electroneutral — Asymmetric anion exchange — Butyrate-gradient-stimulated Cl uptake — Cl-SCFA exchange

Correspondence to: H.J. Binder

Introduction

Short chain fatty acids (SCFA)[1] produced by the colonic microflora are avidly absorbed in the large intestine and also stimulate colonic Na and Cl absorption [1, 4, 5]. Although SCFA absorption has been studied extensively by both in vivo and in vitro methods, a consensus model of colonic SCFA absorption has not evolved. Studies of SCFA absorption in ruminal epithelia and in vivo experiments in the large intestine have been interpreted to indicate that the mechanism of SCFA absorption is nonionic diffusion [6, 13, 17, 19, 20, 24, 25, 28]. In many of these studies, SCFA absorption is accompanied by an increase in luminal bicarbonate; an observation that is also consistent with a SCFA-HCO_3 exchange mechanism [19]. Studies from this laboratory, which were performed across isolated rat colonic mucosa under voltage clamp conditions, demonstrated that butyrate (used as a model SCFA) stimulated active Na and Cl absorption and proposed a model of nonionic diffusion of protonated butyrate across the apical membrane, with its subsequent intracellular dissociation to protons and ionized butyrate and then recycling across the apical membrane via parallel Na-H and Cl-butyrate exchanges, respectively [2, 3]. Such a model would result in overall electroneutral Na-Cl absorption and is similar to models that have been proposed to explain the coupling of Na and Cl absorption by butyrate and formate in guinea pig gallbladder and rabbit proximal tubule, respectively [14, 18]. Thus, this model requires (i) a mechanism of butyrate uptake across the apical membrane (e.g., butyrate-HCO_3 ex-

[1] *Abbreviations used:* AMV, apical membrane vesicles; BLMV, basolateral membrane vesicles; DIDS, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid; FCCP, carbonyl cyanide *p*-(trifluoromethoxy)-phenylhydrazone; MES, 1-[*N*-morpholino]ethanesulfonic acid; NMG, *N*-methyl-D-glucamine; SCFA, short chain fatty acid.

change and/or nonionic butyrate diffusion); (ii) a functional Na-H exchange; and (iii) Cl-butyrate exchange.

Recent studies performed with apical membrane vesicles (AMV) isolated from the rat distal colon and human ileum demonstrated an outward bicarbonate-gradient-stimulated ^{14}C-butyrate uptake (i.e., butyrate-HCO_3 exchange) but did not identify stimulation of ^{14}C-butyrate uptake by an outward Cl gradient (i.e., Cl-butyrate exchange) [12, 16]. Preliminary experiments that were designed to delineate the mechanisms of apical membrane Cl-anion exchange suggested that an outward butyrate gradient stimulated ^{36}Cl uptake [21]. As a result, the present studies were initiated to explore in depth the mechanism of Cl-butyrate exchange in apical membranes of rat distal colon.

Materials and Methods

Apical Membrane Vesicles Preparation

AMV was prepared from normal rat (200–250 g, Sprague Dawley) distal colon by the method of Stieger, Marker and Hauri [27], as described earlier [22]. Purity of the AMV was periodically assessed by 9–12-fold enrichment of K-activated ATPase activity compared to homogenate [8]. AMV were preloaded in an incubation medium (described in the figure legends) and were immediately stored at −70°C. At the time of the experiments, AMV were thawed and incubated at room temperature for at least 30 min.

ATPase Assay

ATPase activity was measured by the method of Forbush [9], as described earlier [8]. Specific activity of K-activated ATPase activity in AMV treated without SDS was 92% of that in AMV with SDS treatment, indicating approximately 92% of the AMV were oriented as right-side-out vesicles. Protein was estimated by the method of Lowry et al. [15].

Uptake Studies

Uptake of ^{36}Cl (New England Nuclear, Boston, MA) was performed at room temperature by rapid filtration technique as described earlier [21]. In brief, outward butyrate gradient-stimulated ^{36}Cl uptake was determined in the AMV preloaded with (mM) 50 Na-butyrate, 100 K-gluconate, 10 NMG-gluconate and 50 MES-Tris (pH 6.5), which were incubated in an incubation medium that contained (mM) 50 Na-gluconate, 100 K-gluconate, 10 NMG-^{36}Cl and 50 MES-Tris (pH 6.5). Uptake was arrested by adding 1 ml ice-cold stop solution. AMV collected on 0.45 μm membrane filters (Millipore, Bedford, MA) were used for radioactive counting.

Appropriate experimental conditions are provided in the figure legends. AMV preincubated for 30 min with 10 μM valinomycin and 100 μM FCCP were used for experiments performed under pH clamp condition. Data presented are the mean values of triplicate assays of a typical experiment. Standard errors less than 5% are not provided in the figures. All experiments were repeated at least three times with different membrane preparations. Kinetic parameters were calculated using the Enzfitter program for IBM PC.

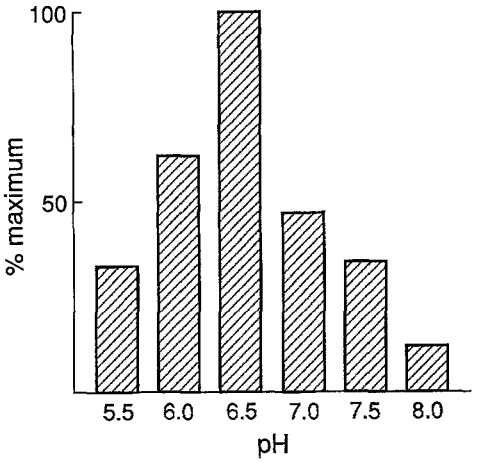

Fig. 1. Effect of pH. AMV were preloaded with (mM) 50 Na-butyrate, 100 K-gluconate, 10 NMG-gluconate and 50 of either MES-Tris (pHs 5.5, 6.0 and 6.5) or HEPES-Tris (pHs 7.0, 7.5 and 8.0). Uptake of ^{36}Cl was measured for 9 sec by incubating AMV in medium that contained 50 mM Na-gluconate, 100 mM K-gluconate, 10 μM valinomycin, 100 μM FCCP, 10 mM NMG-^{36}Cl and 50 mM of either MES-Tris or HEPES-Tris with a pH identical to that of the intravesicular medium. Absolute values presented are DIDS-sensitive uptake that was calculated by subtracting the uptake obtained in the presence of 1 mM DIDS from that of total uptake. Uptake at pH 6.5 considered 100%.

Experiments characterizing butyrate gradient-stimulated ^{36}Cl uptake were performed for 9 sec as the butyrate gradient-stimulated ^{36}Cl uptake was linear for up to at least 10 sec (*data not shown*). The rate of butyrate gradient-stimulated ^{36}Cl uptake was determined at different pHs in the absence of pH gradient (i.e., $pH_{out} = pH_{in}$). As shown in Fig. 1, butyrate gradient-stimulated ^{36}Cl uptake was maximum at pH 6.5. Increasing and decreasing both intravesicular and extravesicular pH from 6.5 progressively reduced the butyrate gradient-stimulated ^{36}Cl uptake (Fig. 1). Thus, the experiments characterizing butyrate gradient-stimulated ^{36}Cl uptake were performed in the absence of a pH gradient at pH 6.5.

Results

An outward butyrate gradient stimulated ^{36}Cl uptake and resulted in a peak uptake at 2 min that was twofold greater than that at equilibrium (Fig. 2). Butyrate gradient-stimulated ^{36}Cl uptake was inhibited completely by 1 mM DIDS, an anion exchange inhibitor, to a rate comparable to that in the absence of a butyrate gradient. Butyrate gradient-stimulated ^{36}Cl uptake was not inhibited by either 1 mM amiloride or ouabain (*data not shown*). These results suggest that butyrate gradient-stimulated ^{36}Cl uptake is an anion exchange process.

DIDS-sensitive Cl-OH exchange has been demonstrated in these AMV [21]. To evaluate the possibility that butyrate gradient-stimulated ^{36}Cl uptake might represent butyrate activation of the Cl-OH exchange, the effect of butyrate on Cl gradient-stimulated ^{36}Cl uptake

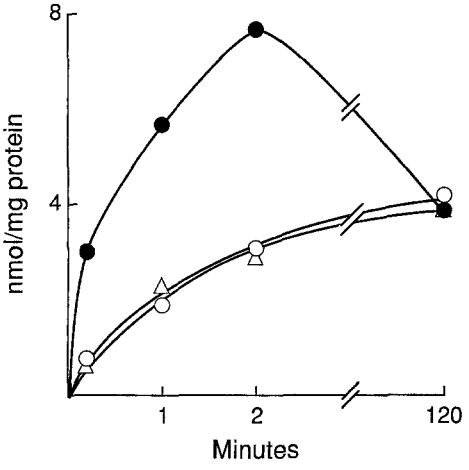

Fig. 2. Effect of outward butyrate gradient. AMV were preloaded with (mM) 50 Na-butyrate, 100 K-gluconate, 10 NMG-gluconate and 50 MES-Tris (pH 6.5). Uptake was measured for 0.2 to 120 min by incubating AMV in medium that contained (mM) 100 K-gluconate, 10 NMG-^{36}Cl, 50 MES-Tris (pH 6.5) and 50 of either Na-butyrate (open circles) or Na-gluconate (filled circles). ^{36}Cl uptake in Na-gluconate was also performed in the presence of 1 mM DIDS (open triangles).

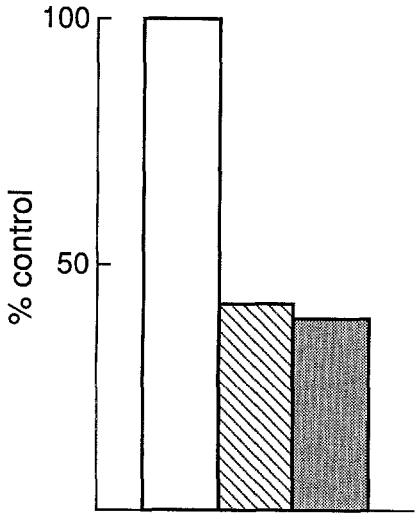

Fig. 3. Effect of butyrate on Cl-Cl exchange. *Open bar:* AMV were preloaded with (mM) 50 NaCl, 100 K-gluconate, 10 NMG-gluconate and 50 MES-Tris (pH 6.5). Uptake of ^{36}Cl was measured for 9 sec by incubating AMV in medium that contained (mM) 50 Na-gluconate, 100 K-gluconate, 10 NMG-^{36}Cl and 50 MES-Tris (pH 6.5). *Hatched bar:* AMV were preloaded with (mM) 50 NaCl, 100 K-gluconate, 10 NMG-gluconate and 50 MES-Tris (pH 6.5). Uptake of ^{36}Cl was measured for 9 sec by incubating AMV in medium that contained (mM) 50 Na-butyrate, 100 K-gluconate, 10 NMG-^{36}Cl and 50 MES-Tris (pH 6.5). *Stippled bar:* AMV were preloaded with (mM) 50 Na-butyrate, 50 KCl, 50 K-gluconate, 10 NMG-gluconate and 50 MES-Tris (pH 6.5). Uptake of ^{36}Cl was measured for 9 sec by incubating AMV in medium that contained (mM) 50 Na-butyrate, 100 K-gluconate, 10 NMG-^{36}Cl and 50 MES-Tris (pH 6.5). Absolute values presented are DIDS-sensitive uptake that was calculated by subtracting the uptake in the presence of 1 mM DIDS from that of total uptake. Uptake obtained in the presence of outward Cl gradient and in the absence of butyrate was considered 100%.

was examined.[2] As shown in Fig. 3, Cl gradient-stimulated ^{36}Cl uptake was inhibited by butyrate to the same degree, whether butyrate was in the extravesicular medium alone or in both the intravesicular and extravesicular medium. These results suggest that butyrate gradient-stimulated ^{36}Cl uptake is not the result of butyrate activation of Cl-OH exchange (i.e., Cl-Cl exchange).

Although the results in Fig. 3 do not demonstrate butyrate activation of Cl-OH exchange, butyrate gradient-stimulated ^{36}Cl uptake could still be the result of the Cl-OH exchange, if outward butyrate movement via nonionic diffusion resulted in an outward hydroxyl gradient (i.e., $pH_{out} < pH_{in}$). Therefore, butyrate gradient-stimulated ^{36}Cl uptake was measured under conditions that prevent the development of a pH gradient. In these experiments, butyrate gradient-stimulated ^{36}Cl uptake was performed both in the presence of potassium and its ionophore valinomycin, and in the presence of FCCP [carbonyl cyanide *p*-(trifluoromethoxy) phenylhydrazone], a proton ionophore, which can function as K-H exchange and dissipate the formation of pH gradients across membrane vesicles. As shown in Fig. 4*A*, the presence of valinomycin and FCCP did not alter butyrate gradient-stimulated ^{36}Cl uptake. In contrast to its effect on butyrate gradient-stimulated ^{36}Cl uptake, valinomycin and FCCP almost completely inhibited outward hydroxyl gradient-stimulated ^{36}Cl uptake (i.e., pH_{out}/pH_{in} = 5.5/7.5) (Fig. 4*B*). These results confirm that butyrate gradient-stimulated ^{36}Cl represents a distinct Cl-butyrate exchange.

The effect of outward Cl gradients on ^{14}C-butyrate uptake and ^{36}Cl uptake was examined. As shown in Fig. 5*A*, ^{14}C-butyrate uptake was not stimulated by an outward Cl gradient, confirming earlier observations [16]. However, ^{36}Cl uptake was substantially stimulated by an outward Cl gradient presumably via Cl-Cl exchange (Fig. 5*B*). The absence of an outward Cl gradient-stimulated ^{14}C-butyrate uptake (Fig. 5*A*) and the presence of outward butyrate gradient-stimulated ^{36}Cl uptake (Fig. 2) in these AMV suggest that intravesicular and extravesicular binding sites of Cl-butyrate exchange may have varying affinities for butyrate and/or Cl.

To establish the relative affinities for butyrate and/or Cl at the intravesicular and extravesicular binding sites of the Cl-butyrate exchange, the stimulation of ^{36}Cl efflux by extravesicular Cl and butyrate was examined (Fig. 6). The experimental design of this study

[2] Cl gradient-stimulated ^{36}Cl uptake (Cl-Cl exchange) could represent Cl-OH, Cl-butyrate or Cl-HCO_3 exchanges. In this experiment Cl gradient-stimulated ^{36}Cl uptake is used as a surrogate for Cl-OH exchange.

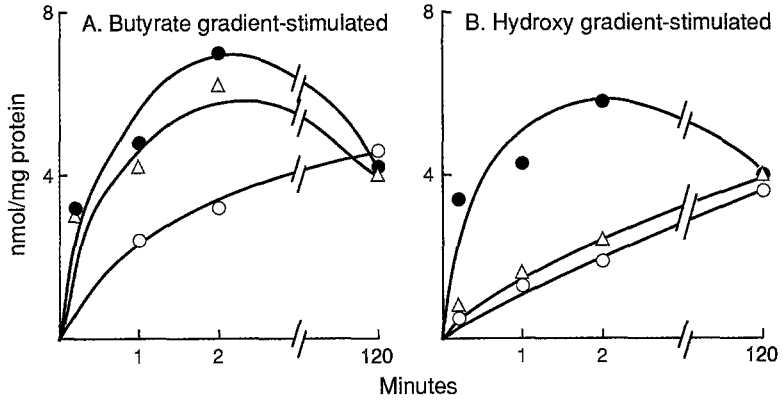

Fig. 4. Effect of valinomycin and FCCP on outward butyrate gradient-stimulated and hydroxyl gradient-stimulated ^{36}Cl uptake. (*A*) AMV were preloaded with (mM) 50 Na-butyrate, 100 K-gluconate, 10 NMG-gluconate and 50 MES-Tris (pH 6.5). Uptake of ^{36}Cl was measured for 0.2–120 min by incubating AMV in medium that contained (mM) 100 K-gluconate, 10 NMG-^{36}Cl, 50 MES-Tris (pH 6.5) and 50 of either Na-butyrate (open circles) or Na-gluconate (filled circles). Uptake with Na-gluconate was also performed in the presence of 10 μM valinomycin and 100 μM FCCP (open triangles). Valinomycin and FCCP were dissolved in ethanol. All medium contained 0.5% ethanol. (*B*) AMV were preloaded with (mM) 150 K-gluconate, 10 NMG-gluconate and 50 MES-Tris (pH 7.5). Uptake of ^{36}Cl was measured for 0.2–120 min by incubating AMV in medium that contained (mM) 150 K-gluconate, 10 NMG-^{36}Cl and 50 of either HEPES-Tris (pH 7.5) (open circles) or MES-Tris (pH 5.5) (filled circles). Uptake with MES-Tris was also performed in the presence of 10 μM valinomycin and 100 μM FCCP. Valinomycin and FCCP were dissolved in ethanol. All medium contained 0.5% ethanol.

included an initial uptake step followed by the efflux experiment. In the uptake step, an outward butyrate gradient stimulated ^{36}Cl uptake during an initial 30 sec incubation period. To determine the rate of ^{36}Cl efflux, the uptake medium containing the vesicles was then diluted tenfold with medium that contained varying concentrations of either Cl or butyrate. As shown in Fig. 6, the rate of ^{36}Cl efflux was accelerated by increasing concentrations of extravesicular Cl and butyrate. Although both extravesicular Cl and butyrate stimulated ^{36}Cl efflux, the effect of 15 mM Cl was sixfold greater than that of 15 mM butyrate. Stimulation of ^{36}Cl efflux by Cl saturated at approximately 30 mM. Both butyrate- and Cl-accelerated ^{36}Cl efflux was completely inhibited by 1 mM DIDS. These results confirm that both butyrate and Cl accelerated ^{36}Cl efflux via a DIDS-sensitive anion exchange process. Demonstration that the rate of stimulation of ^{36}Cl efflux by extravesicular butyrate was fourfold lower than that by Cl is consistent with the postulate that the relative affinity of the extravesicular binding site of the Cl-butyrate exchange for butyrate is lower than that of the intravesicular binding site.

The effect of outward gradients of various SCFA on ^{36}Cl uptake was examined to determine the specificity of Cl-butyrate exchange. As shown in Fig. 7, outward gradients of butyrate, propionate, acetate and formate all stimulated the DIDS-sensitive fraction of ^{36}Cl uptake. DIDS inhibited ^{36}Cl uptake stimulated by an outward gradient of these four SCFAs by approximately 60%. The DIDS-insensitive fractions of ^{36}Cl uptake were identical. These results suggest that Cl-butyrate exchange has affinity for several SCFAs.

Kinetic studies were performed to provide further charactemrization of Cl-butyrate exchange as a carrier-mediated anion exchange process (Figs. 8 and 9). As shown in Fig. 8*A* and *B*, both increasing extravesicular Cl concentrations and increasing intravesicular butyrate concentration resulted in an increase of and saturation of ^{36}Cl uptake. Analysis of these data with Lineweaver-Burk plot yielded kinetic constants: apparent K_m for extravesicular Cl was 26.8 ± 3.4 mM and V_{max} was 12.4 ± 0.6 nmol/mg protein · 9 sec; while apparent K_m for intravesicular butyrate was 5.9 ± 1.4 mM and V_{max} for Cl was 5.9 ± 0.3 nmol/mg protein · 9 sec. Increasing DIDS concentration progressively inhibited butyrate gradient-stimulated ^{36}Cl uptake with a half-maximal inhibitory concentration (IC_{50}) of approximately 68.4 ± 6.2 μM (Fig. 9). The results confirm Cl-butyrate exchange is a DIDS-sensitive carrier-mediated anion exchange.

Discussion

SCFAs are synthesized in the lumen of the mammalian large intestine from nonabsorbed carbohydrate by colonic bacterial enzymes. In contrast to carbohydrates (i.e., starch, disaccharides and monosaccharides) that are not absorbed in the colon, SCFA are absorbed in the colon in substantial amounts and also stimulate colonic fluid, Na and Cl absorption. As a result, SCFA ab-

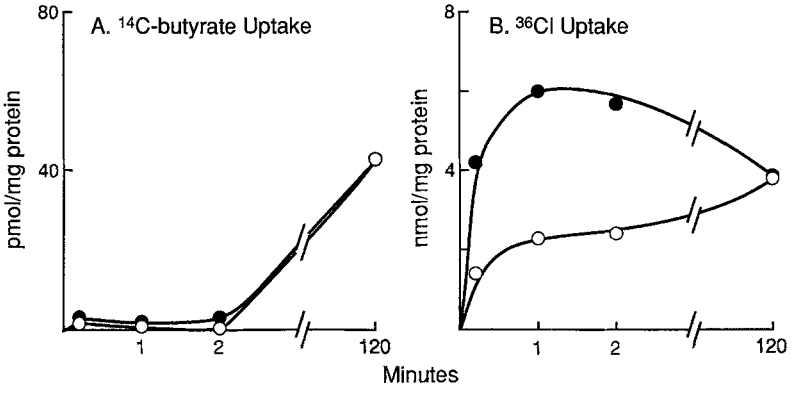

Fig. 5. Effect of outward Cl gradients on ^{14}C-butyrate uptake and ^{36}Cl uptake. AMV were preloaded with (mM) 50 KCl, 100 K-gluconate, 10 NMG-gluconate and 50 MES-Tris (pH 6.5). (*A*) Uptake of ^{14}C-butyrate was measured for 0.2–120 min by incubating AMV in medium that contained either (mM) 50 KCl, 100 K-gluconate, 10 NMG-gluconate, 0.5 ^{14}C-butyrate and 50 MES-Tris (pH 6.5) (open circles) or 150 mM K-gluconate, 10 mM NMG-gluconate, 500 μM ^{14}C-butyrate and 50 mM MES-Tris (pH 6.5) (filled circles). All medium contained 10 μM valinomycin, 100 μM FCCP and 0.5% ethanol. (*B*) Uptake of ^{36}Cl was measured for 0.2–120 min by incubating AMV in medium that contained either (mM) 50 KCl, 100 K-gluconate, 10 NMG-^{36}Cl and 50 MES-Tris (pH 6.5) (open circles) or 150 K-gluconate, 10 NMG-^{36}Cl and 50 MES-Tris (pH 6.5) (filled circles). All medium contained 10 μM valinomycin, 100 μM FCCP and 0.5% ethanol.

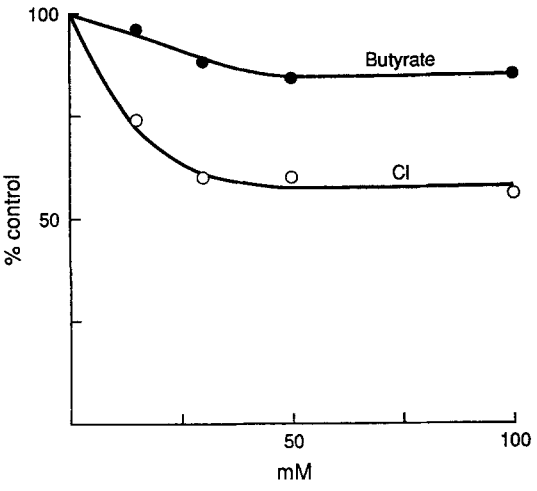

Fig. 6. Effect of extravesicular butyrate and Cl on ^{36}Cl efflux. AMV were preloaded with (mM) 50 Na butyrate, 100 Na-gluconate, 10 NMG-gluconate and 50 MES-Tris (pH 6.5). Uptake of ^{36}Cl was performed for 30 sec by diluting the AMV in medium that contained (mM) 150 Na-gluconate, 50 NMG-^{36}Cl and 50 MES-Tris (pH 6.5). Efflux of ^{36}Cl was determined for 9 sec by incubating the mixture containing AMV in medium with 10 mM NMG-gluconate and 50 mM MES-Tris (pH 6.5) and varying concentrations of either NaCl (open circles) or Na-butyrate (filled circles). Isosmolarity was maintained by adjusting Na-gluconate concentration. Experiments were also performed in the presence of 1 mM DIDS. Absolute values presented are DIDS-sensitive efflux that was calculated by subtracting the efflux in the presence of 1 mM DIDS from total efflux. Vesicular ^{36}Cl content at 30 sec uptake was considered 100%.

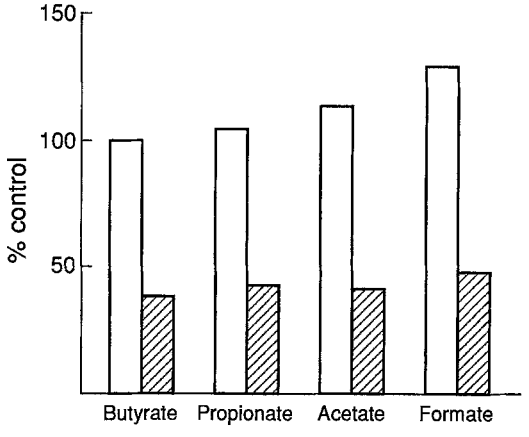

Fig. 7. Effect of various outward SCFA gradient on ^{36}Cl uptake. AMV were preloaded with (mM) 50 K-salt of indicated fatty acid (except Na-butyrate), 100 K-gluconate, 10 NMG-gluconate and 50 MES-Tris (pH 6.5). Uptake of ^{36}Cl was measured for 9 sec by incubating the AMV in medium that contained (mM) 150 K-gluconate, 10 NMG-^{36}Cl and 50 MES-Tris (pH 6.5) (open bars). Experiments were also performed in the presence of 1 mM DIDS (hatched bars). Butyrate gradient-stimulated ^{36}Cl uptake was considered 100%.

sorption represents an excellent adaptive mechanism to compensate for incomplete small intestinal absorption of carbohydrate [1, 4, 5, 6, 13, 17, 19, 20, 24, 25, 28]. In vitro studies performed under voltage clamp conditions in rat distal colon resulted in a model of SCFA (butyrate) stimulation of electroneutral Na and Cl absorption that proposed apical membrane butyrate uptake was coupled to a functional Na-H exchange and a Cl-SCFA exchange in rat distal colon [2, 3]. Although 99% of SCFA exist as anions at the pH (6.9) in the luminal microclimate, previous studies emphasized nonionic diffusion as the probable mechanism for SCFA absorption [1, 2, 4, 5]. However, recent studies with AMV isolated from rat distal colon, and from human

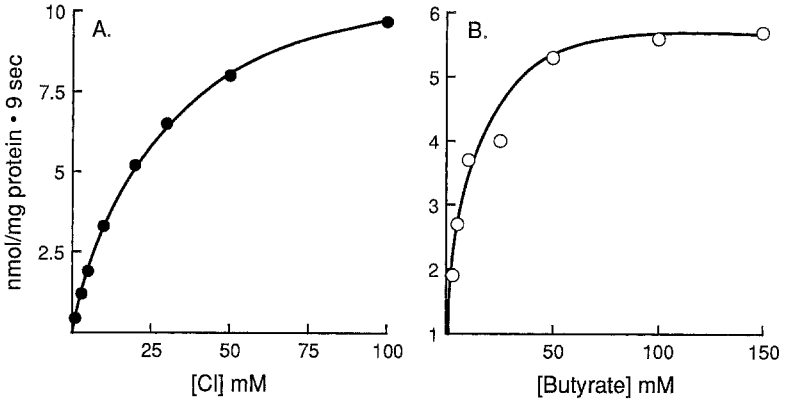

Fig. 8. (*A*) Effect of extravesicular Cl concentration on butyrate gradient-stimulated ^{36}Cl uptake. AMV were preloaded with (mM) 50 Na-butyrate, 100 K-gluconate, 10 NMG-gluconate and 50 MES-Tris (pH 6.5). Uptake of ^{36}Cl was measured for 9 sec by incubating the AMV in medium that contained (mM) 50 Na-gluconate, trace of NMG-^{36}Cl, 50 MES-Tris (pH 6.5) and varying concentration of KCl. Isosmolarity of the medium was maintained by varying concentration of NMG-gluconate and K-gluconate. Absolute values presented are DIDS-sensitive uptake that was calculated by subtracting the uptake in the presence of 1 mM DIDS from total uptake. (*B*) Effect of intravesicular butyrate concentration. AMV were preloaded with (mM) 100 K-gluconate, 10 NMG-gluconate, 50 MES-Tris (pH 6.5) and varying concentrations of Na-butyrate (3–200 mM) and Na-gluconate (147–0 mM). Uptake of ^{36}Cl was measured for 9 sec by incubating the AMV in medium that contained (mM) 100 K-gluconate, 200 Na-gluconate, 10 NMG^{36}Cl and 50 MES-Tris (pH 6.5). Absolute values presented are DIDS-sensitive uptake, calculated by subtracting the uptake in the presence of 1 mM DIDS from total uptake.

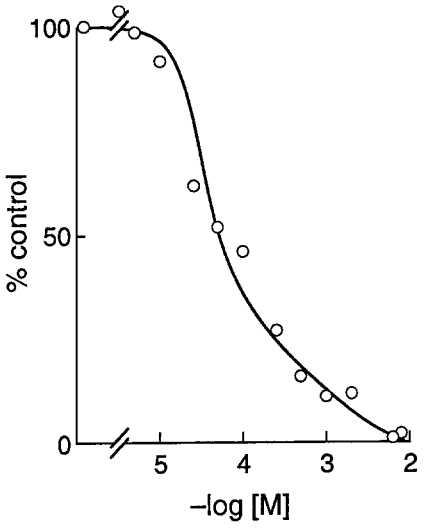

Fig. 9. Effect of DIDS concentration. AMV were preloaded with (mM) 50 Na-butyrate, 100 K-gluconate, 10 NMG-gluconate and 50 MES-Tris (pH 6.5). Uptake of ^{36}Cl was measured for 9 sec by incubating the AMV in medium that contained (mM) 50 Na-gluconate, 100 K-gluconate, 10 NMG^{36}Cl, 50 MES-Tris (pH 6.5) and varying concentrations of DIDS. Absolute values presented are calculated after subtraction of uptake obtained in the absence of butyrate gradient from that in the presence of butyrate gradient.

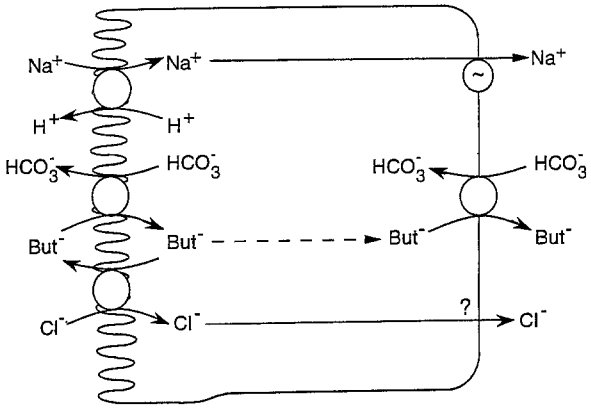

Fig. 10. Model of butyrate stimulation of Na and Cl absorption (*see text* for details).

ileum and colon demonstrated that an outward bicarbonate gradient stimulated butyrate uptake and concluded that butyrate-HCO_3 exchange was the primary mechanism for SCFA absorption [11, 12, 16]. These AMV studies did not identify either outward Cl gradient-stimulated butyrate uptake (i.e., Cl-SCFA exchange) or inward acid pH gradient-stimulated butyrate uptake (i.e., nonionic butyrate diffusion) [11, 12, 16]. As a result, those observations did not support the cellular model proposed for SCFA absorption and its stimulation of electroneutral Na-Cl absorption in rat distal colon. Thus, the present study with AMV was initiated to address the possibility that the failure to observe Cl gradient-stimulated ^{14}C-butyrate uptake was a consequence of the Cl-butyrate exchange manifesting differing affinities for butyrate at the intravesicular and extravesicular binding sites.

The present investigation reported that outward butyrate gradient stimulated Cl uptake via an electroneu-

tral, carrier-mediated anion exchange (i.e., Cl-butyrate exchange) process. This model is supported by the observations that an outward butyrate gradient-stimulated Cl uptake was: (i) not inhibited by pH and/or voltage clamping (Fig. 4*A*); (ii) saturated by both increasing extravesicular Cl concentration (K_m for Cl was 26.8 ± 3.4 mM) and increasing intravesicular butyrate concentration (K_m for butyrate was 5.9 ± 1.4 mM) (Fig. 8*A* and *B*); and inhibited by DIDS with a IC_{50} of 68.4 ± 6.5 μM (Fig. 9).

These present experiments provide excellent evidence that there are at least three different Cl-anion exchanges in this AMV preparation: Cl-OH, Cl-HCO_3, and Cl-SCFA exchanges. It is possible that these anion exchanges present in AMV might represent apical membranes that were isolated from more than one cell type in that distal colonic epithelia consist of at least four different cell types. Cl-butyrate exchange differs from Cl-HCO_3 exchange, as the kinetic constants significantly differ (i.e., apparent K_m for Cl is 26.4 and 10.2 mM, respectively, for these two exchanges; while IC_{50} for DIDS is approximately 68 and 7.6 μM, for Cl-butyrate exchange and Cl-HCO_3 exchange, respectively) (Figs. 8*A* and 9) [21]. Although the kinetic constant for Cl is almost similar for Cl-butyrate exchange and for Cl-OH exchange, the former is pH gradient independent, while the latter is pH gradient dependent in that it is inhibited by pH clamping (Fig. 3).

Butyrate movement across AMV occurs via at least two different anion exchanges: butyrate-HCO_3 exchange and Cl-butyrate exchange. It is unlikely that these two butyrate anion exchanges represent the same transport proteins since Cl-butyrate exchange is DIDS sensitive while butyrate-HCO_3 exchange is not inhibited by DIDS. The DIDS-insensitive butyrate-butyrate exchange that presumably represents butyrate-HCO_3 exchange was not altered when studied at different pHs [16], while butyrate-Cl exchange was greater at pH 6.5 compared to that at lower and higher pHs (Fig. 1). These results are consistent with an earlier observation in which Crump et al. [7] observed acetate-absorption-coupled bicarbonate secretion at lower luminal pH (i.e., at pH 6.4) which disappeared at pH 7.4. Thus, it appears that Cl-SCFA exchange is activated by low pH.

The model proposed to explain butyrate stimulation of electroneutral NaCl absorption includes apical membrane butyrate-HCO_3 and Cl-butyrate exchanges and partial recycling of butyrate across the apical membrane [2, 3]. These anion exchanges appear to represent a specialized apical membrane transport system in that the apical membrane butyrate-HCO_3 exchange [16] is distinct from the butyrate-HCO_3 exchange of basolateral membrane [23] and Cl-butyrate exchange is not expressed in BLMV (*unpublished observations*).

An outward butyrate gradient stimulated the Cl uptake, but an outward Cl gradient did not stimulate the butyrate uptake (Figs. 2 and 5*A*). These results are consistent with the intravesicular and extravesicular binding sites of butyrate-Cl exchange that have different relative affinities for butyrate. This possibility was supported by the observations that extravesicular Cl induced a substantially greater rate of Cl efflux than extravesicular butyrate (Fig. 6). We conclude from these results that the intravesicular binding site has a higher affinity for butyrate than the extravesicular binding site and is responsible for the rectification of butyrate movement across the apical membrane and Cl movement into the cell. Although ion exchanges must be thermodynamically symmetrical, they may possess varying affinities for different ions at their respective binding sites. Such phenomena have been well established for anion exchanges in erythrocyte membrane [10, 26]. This observation would be consistent with stimulation of Na-Cl absorption by butyrate or by its analogues (e.g., propionate, acetate and formate). It is of interest that although a formate gradient stimulates Cl uptake (Fig. 7) indicating the presence of Cl-formate exchange, formate does not enhance electroneutral Na-Cl absorption [2]. The failure of formate to stimulate Na-Cl absorption is not a consequence of the absence of a Cl-formate exchange but may be a result of impaired apical formate uptake. Recent studies demonstrated that formate did not inhibit bicarbonate gradient-stimulated ^{14}C-butyrate AMV (*unpublished observations*).

In conclusion, we propose a model in which SCFA absorption occurs via an apical SCFA-HCO_3 exchange (Fig. 10). HCO_3 efflux as a consequence of butyrate uptake would most likely decrease intracellular pH, and maximal Cl-butyrate exchange activity is observed at pH 6.5 (Fig. 1). This decrease in intracellular pH would also stimulate Na-H exchange resulting both in Na uptake and a return of intracellular pH to resting levels (Fig. 10). The overall effect will be enhancement of electroneutral Na-Cl absorption.

This study was supported in part by a Public Health Service research grant (DK 14669) provided by the National Institute of Diabetes, Digestive and Kidney Diseases. Ms. Mary Guidone provided excellent secretarial assistance.

References

1. Bergman, E.N. 1990. Energy contribution of volatile fatty acids from the gastrointestinal tract in various species. *Physiol. Rev.* **70**:567–590
2. Binder, H.J., Mehta, P. 1989. Short chain fatty acids stimulate active sodium and chloride absorption in vitro in the rat distal colon. *Gastroenterology* **96**:989–996
3. Binder, H.J., Mehta, P. 1990. Characterization of butyrate-dependent electroneutral Na-Cl absorption in the rat distal colon. *Pfluegers Arch.* **417**:365–369
4. Binder, H.J., Sandle, G.I., Rajendran, V.M. 1991. Colonic fluid and electrolyte transport in health and disease. *In:* The Large In-

testine: Physiology, Pathophysiology and Disease. S.F. Phillips, J.H. Pemberton, and R.G. Shorter, editors. pp. 141–168. Raven, New York

5. Bugaut, M. 1987. Occurrence, absorption and metabolism of short chain fatty acids in the digestive tract of mammals. *Comp. Biochem. Physiol.* **86B:**439–472
6. Cummings, J.H. 1981. Short chain fatty acids in the human colon. *Gut* **22:**763–769
7. Crump, M.H., Argenzio, R.A., Whipp, S.C. 1980. Effects of acetate on absorption of solute and water from the pig colon. *Am. J. Vet. Res.* **41:**1565–1568
8. Del Castillo, J.R., Rajendran, V.M., Binder, H.J. 1991. Apical localization of ouabain-sensitive K^+-activated ATPase activities in rat distal colon. *Am. J. Physiol.* **261:**G1005–G1011
9. Forbush, B., III. 1993. Assay of Na,K-ATPase in plasma membrane preparations: increasing the permeability of membrane vesicles using sodium dodecyl sulphate buffered with bovine serum albumin. *Anal. Biochem.* **128:**159–163
10. Gunn, R.B., Frohlich, O. 1979. Asymmetry in the mechanism for anion exchange in human red blood cell membranes. Evidence for reciprocating sites that react with one transported anion at a time. *J. Gen. Physiol.* **74:**351–374
11. Harig, J.M., Knaup, S.M., Shoshara, J., Dudeja, P.K., Ramaswamy, K., Brasitus, T.A. 1990. Transport of *n*-butyrate into human colonic luminal membrane vesicles. *Gastroenterology* **98:**553*A* (*Abstr.*)
12. Harig, J.M., Soergel, K.H., Barry, J.A., Ramaswamy, K. 1991. Transport of propionate by human ileal brush-border membrane vesicles. *Am. J. Physiol.* **260:**G776–G782
13. Hennings, S.J., Hird, F.J.R. 1972. Transport of acetate and butyrate in the hind gut of the rabbit. *Biochem. J.* **130:**791–796
14. Karniski, L.P., Aronson, P.S. 1985. Chloride/formate exchange with formic acid recycling: a mechanism of active chloride transport across epithelial membranes. *Proc. Natl. Acad. Sci. USA* **82:** 6362–6365
15. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193:**265–275
16. Mascolo, N., Rajendran, V.M., Binder, H.J. 1991. Mechanism of short-chain fatty acid uptake by apical membrane vesicles of rat distal colon. *Gastroenterology* **101:**331–338
17. McNeil, N.I., Cummings, J.H., James, W.P.T. 1979. Rectal absorption of short chain fatty acids in the absence of chloride. *Gut* **20:**400–403
18. Petersen, K.-U., Wood, J.R., Schulze, G., Heintze, K. 1981. Stimulation of gallbladder fluid and electrolyte absorption by butyrate. *J. Membrane Biol.* **62:**183–193
19. Powell, D.W. 1986. Ion and water transport in the intestine. *In:* Physiology of Membrane Disorders. T.E. Andreoli, J.F. Hoffman, D.D. Fanestil, S.G. Schultz, editors. 2nd ed. pp. 559–596. Plenum, New York
20. Rabbani, G., Binder, H.J. 1989. Evidence of active butyrate absorption by rat distal colon. *Acta Vet. Scand.* **86:**195 (Abstr.)
21. Rajendran, V.M., Binder, H.J. 1993. Cl-HCO_3 and Cl-OH exchanges mediate Cl uptake in apical membrane vesicles of rat distal colon. *Am. J. Physiol.* **264:**G874–G879
22. Rajendran, V.M., Kashgarian, M., Binder, H.J. 1989. Aldosterone induction of electrogenic sodium transport in the apical membrane vesicles of rat distal colon. *J. Biol. Chem.* **264:**18638–18644
23. Reynolds, D.A., Rajendran, V.M., Binder, H.J. 1993. Bicarbonate-stimulated [^{14}C]butyrate uptake in basolateral membrane vesicles of rat distal colon. *Gastroenterology* **105:**725–732
24. Rubsamen, K., Engelhardt, W.V. 1981. Absorption of Na, H ions and short chain fatty acids from sheep colon. *Pfluegers Arch.* **391:** 141–146
25. Ruppin, H., Bar-Meir, S., Soergel, K., Wood, C.M., Schmitt, M.G., Jr. 1980. Absorption of short chain fatty acids by the colon. *Gastroenterology* **78:**1500–1507
26. Sanders, D., Hansen, U.P., Gradmann, D., Slayman, C.L. 1984. Generalized kinetic analysis of ion-driven cotransport systems: A unified interpretation of selective ionic effects on Michaelis parameters. *J. Membrane Biol.* **77:**123–152
27. Stieger, B., Marker, A., Hauri, H.P. 1986. Isolation of brush border membranes from rat and rabbit colonocytes: Is alkaline phosphatase a marker enzyme? *J. Membrane Biol.* **91:**19–31
28. Venary, M., Marty, J. 1984. Absorption and metabolism of butyric acid in rabbit hind gut. *Comp. Biochem. Physiol.* **77:**89–96