SCFAs: The Enigma of Weak Electrolyte Transport in the Colon

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Short-chain fatty acids are the predominant luminal anions in the colon (>75 mM) and thus create a rather unique environment for transporting epithelium. The colon absorbs short-chain fatty acids, either by diffusion of the protonated species across the apical membrane or by an anion exchange process with bicarbonate. Additionally, short-chain fatty acids modulate Na absorption, Cl secretion, intracellular pH, and cell volume.

Over the last twenty years, the putative role of short-chain fatty acids (SCFAs) has been transformed from that of a culprit in the pathogenesis of carbohydrate-induced diarrheas to a pluripotential benefactor of multiple epithelial functions. Clues from rumen physiologists, who correctly recognized that SCFAs were rapidly absorbed across the epithelium, and from scientists involved in tissue culture, who identified SCFAs as potent modifiers of a wide variety of cancer cell lines, led to a reassessment of the role of SCFAs in epithelial physiology. At present, multiple potential roles of SCFAs are being actively investigated, including: 1) how SCFAs are transported across epithelia; 2) how SCFAs alter other transport functions, including intracellular pH (pHi) and cell volume regulation; 3) the nutrient role of SCFAs; 4) the array of SCFA...
effects as a biological modifier; and, finally, 5) SCFAs as potential therapeutic agents.

The clinical use of SCFAs to treat inflammatory diseases of the colon has led to an increased interest in their effects on the colonic epithelium and, more generally, the role of luminal factors in modifying epithelial function. The initial application of SCFAs as therapy for inflammation arose from a recognition that they were the preferred metabolic fuel for the colonic epithelial cell and provided as much as 15% of daily caloric requirements in humans. The role of SCFAs as biological modifiers is beyond the scope of this review; however, SCFAs, particularly butyrate, may have important roles in regulating proliferation, differentiation, gene expression, immune regulation, and wound healing in the colon.

**What are SCFAs and where do they come from?**

SCFAs are 2- to 5-carbon weak acids (pK 4.8) arising from bacterial metabolism of primarily carbohydrate but also protein. In most animals, this occurs when unabsorbed carbohydrate leaves the relatively sterile environment of the small bowel, crosses the ileocecal valve, and confronts colonic bacteria. The principal SCFAs found in the colonic lumen are acetate, propionate, and butyrate. They are the predominant luminal anions in colonic fluid, with a normal concentration range of 70–100 mM and a relative ratio of 60 acetate:25 propionate:15 butyrate. Although there are differences in their roles as nutrients and biological modifiers, SCFAs have fairly similar effects on transport.

SCFA production is a reflection of diet; although it is now clear that there is a low-level, normal rate of “malabsorption” of most carbohydrates, diets high in fiber, beans, resistant starches, and complex carbohydrates generally lead to a greater rate of SCFA formation. With recognition of the beneficial effects of dietary fiber, many investigators have hypothesized that these effects are linked to SCFAs.

Defining the role of SCFAs is complicated by two factors: metabolism and acid-base equilibria. Because SCFAs are the preferred metabolic substrate for the colonic epithelium, it is difficult to distinguish a SCFA effect on a specific transporter from a more generalized or unexpected metabolic effect. To avoid these complexities, investigators often employ SCFAs that are not as avidly metabolized by the colon, such as propionate and/or isobutyrate.

Because SCFAs are weak electrolytes, there is an equilibrium between the protonated form (HA) and the anion (A⁻). At physiological pH, ~99% of SCFAs will be in the A⁻ form. In contrast to HA, which is lipid soluble and therefore readily diffuses through cell membranes, A⁻ requires specific transport proteins to cross into or out of a cell. Equilibria are necessarily established within and between the different compartments of the epithelium, i.e., luminal, intracellular, and basolateral. Subtle changes in one compartment will be reflected in changes in other compartments. Thus, when one species of SCFA is transported into or out of a cell, it will affect the concentration of another. Minor changes in pH across membranes may have a profound effect on the relative distribution of SCFAs. These factors have plagued the attempts of transport physiologists to clarify the mechanisms of SCFA movement across epithelia.

**SCFA transport**

**Apical uptake.** In vivo, SCFAs are rapidly absorbed and stimulate both Na absorption and bicarbonate secretion. The estimated daily SCFA absorption from the human colon is roughly equivalent to colonic Na absorption (300 mmol), suggesting that transport of this solute is integral to the absorptive functions of the colon.

In vitro studies have suggested several different mechanisms for SCFA absorption (Fig. 1). Although the high luminal concentration of SCFAs creates a significant lumen-to-serosa gradient, there is minimal paracellular diffusion. Thus investigators have focused on transcellular mechanisms of absorption.

Transporters that provide luminal protons stimulate SCFA absorption. In proximal colon, stimulation of Na/H exchange across the apical membrane and, in turn, electroneutral Na absorption by epinephrine increases SCFA absorption (14). Inhibition of Na/H exchangers (NHE) by amiloride or theophylline causes a parallel decrease in SCFA absorption. In distal colon, the same correlation exists between activity of the K⁺-H⁺ATPase and SCFA absorption (7).

Additionally, a physiological pH gradient across the mucosa can drive SCFA absorption. Acid-base equilibrium theory predicts that SCFAs, faced with a pH gradient, tend to distribute toward the compartments with a higher pH. This occurs with modest mucosal acidification in both sheep rumen and rabbit colon (13). Diffusive entry of protonated SCFAs will increase with increasing chain length (i.e., increased lipid solubility). This is seen with many (5) but not all experimental models. These observations are most consistent with diffusive entry of a protonated SCFA across the apical membrane driven by pH or proton gradients.

“SCFA production is a reflection of diet…”
Studies on apical membrane vesicles from small intestine and colon have found evidence for a family of anion exchangers that mediate SCFA-/HCO₃⁻ exchange (9). In addition, a relatively unique anion exchanger that mediates rectified SCFA-/Cl⁻ exchange in colonic apical membranes has been described. These exchangers have been inconsistently inhibited by relatively high concentrations of the stilbene 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS). Methodological questions about rapid equilibration of the nonionized SCFA, maintenance of transvesicular pH gradient, and the use of nonphysiological bicarbonate gradients need to be fully resolved before one can accurately assess the biological importance of these anion exchangers.

Integral to characterizing a specific transport process is the identification of inhibitors and competitors. As noted above, the all-purpose anion exchange inhibitor DIDS, even at high concentrations, does not consistently block SCFA/HCO₃⁻ exchange in vesicles. However, other SCFAs may inhibit SCFA/HCO₃⁻ exchanger activity. Mercaptopropionate does inhibit butyrate uptake in apical membrane vesicles from rat distal colon. However, mercaptopropionate elicits a distinct pattern of profound cellular acidification, different from those of the “conventional” SCFAs; it is unclear whether the inhibition may be caused by the differential effect on intracellular pH rather than a specific blocking of a carrier-mediated mechanism.

Compared with vesicle studies, observations in Ussing chambers do not provide definitive evidence for anion exchangers. No bicarbonate-dependent SCFA absorption is observed in rabbit colon with the Ussing chamber technique, nor does DIDS block SCFA absorption (13, 14); however, there is some HCO₃⁻-dependent SCFA absorption in the guinea pig (8). More detailed studies will be needed to define the relative importance of diffusion of protonated SCFAs compared with anion exchanger-mediated transport in specific epithelia.

Basolateral processes. As with most absorptive processes, investigators have focused more on apical entry than the basolateral exit step. The fate of SCFAs, once inside the cell, is complex but to a large extent unstudied. Given the equilibria established between lumen and cell, the intracellular concentration of SCFAs may be quite high, perhaps >50 mM. Limited apical permeability and cellular metabolism would undoubtedly decrease that concentration. Transcellular transport would also limit cellular accumulation.

One potential basolateral transporter is a SCFA/HCO₃⁻ exchanger similar to the apical anion exchanger. However, because of the physiological gradients from mucosa to serosa, high intracellular SCFA concentrations may permit this exchanger to export SCFAs from the cell, i.e., in a different direction from the apical exchanger (10).

Another possible mechanism for SCFA and basolateral exit may involve metabolism of SCFAs to HCO₃⁻. Although most studies demonstrate electroneutral transport of SCFAs, mucosal SCFAs produce a concentration-dependent decrease in short-circuit current in rat colon associated with a stimulation of NaCl absorption. This change is dependent on Cl⁻, diminished by a Cl⁻-channel blocker, partially inhibited by HCO₃⁻ removal, and correlated with SCFA metabolism (6). This suggests that intracellular metabolism of SCFAs may lead to increased production of HCO₃⁻ with subsequent stimulation of apical Cl⁻/HCO₃⁻ exchange and possibly basolateral volume-sensitive Cl⁻ channels.

Because SCFAs and bicarbonate have similar effects on transport in different epithelia, they may share a common pathway across the basolateral membrane. One possible transporter is the Na-HCO₃ symport described in renal epithe-
lia and, more recently, in rabbit proximal colon. In rat colon, in a HCO$_3^-$-Cl-free environment, mucosal SCFAs stimulate Na$^+$ absorption, decrease the short-circuit current, and decrease bicarbonate secretion. A basolateral Na-HCO$_3^-$ or Na-SCFA cotransporter may be responsible for these observations.

SCFAs as regulators of other transport functions

In addition to their own transport, whether through diffusive flux or anion exchangers, SCFAs also may modify several other membrane transport functions. Epithelial cells exposed to SCFAs undergo an increase in their cell volume and a decrease in pH$_i$. These events then elicit a series of homeostatic events to return volume and pH$_i$ to baseline (Fig. 2). Both the initial reaction and the regulatory response involve specific transporters and the movement of other solutes and electrolytes. In general, similar models can explain the initial effect of SCFAs on pH$_i$ and volume; however, there are critical differences and some inconsistencies in modeling the recovery phases for pH$_i$ and volume.

The consensus model of SCFA-induced acidification describes diffusive entry of the protonated species (HA) into the cell, dissociation, and release of H$^+$. Longer SCFAs have a more potent acidifying effect on isolated colonocytes (5). In Na-free media, the extent of cellular acidification is selectively altered by changes in the protonated, not the ionized, species of SCFA (2). These observations are consistent with diffusive entry. However, there does appear to be a “saturation” effect of acidification at high SCFA concentrations (2, 5). Whether this represents saturation of an unidentified transporter or a secondary response of the cell is unclear.

The colonocyte adapts to acidification by numerous regulatory mechanisms to return toward the resting pH$_i$. Perhaps the most important are NHEs. Amiloride-induced inhibition of NHE slows, but does not eliminate, pH$_i$ recovery. There is an additional Na-independent pH$_i$ recovery mechanism, which appears to be inhibited by lowering extracellular A$^-$ and/or extracellular pH, suggesting that the Na-independent pH$_i$ recovery may represent SCFA anion entry into the cell (2). Because most pH$_i$ recovery processes are associated with proton extrusion from the cell, this model of base entry is rather unique. Cinnamate, an inhibitor of proton-monocarboxylate transport, has been shown by some, but not others, to also have a role in pH$_i$ recovery.

Although other organic anions such as lactate and formate acidify colonocytes, they do not elicit the same homeostatic pH$_i$ responses as SCFAs. The recovery after a lactate challenge is prolonged, suggesting that anion-dependent factors, rather than acidification alone, determine the recovery. The mechanisms for this are unclear. However, because fecal lactate and SCFA concentrations often exhibit a reciprocal relationship, these differences may be physiologically important.

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"...most pH$_i$ recovery processes are associated with proton extrusion...."

FIGURE 2. Potential intracellular effects of SCFAs. Although SCFAs stimulate Na absorption, decrease pH$_i$, and increase cell volume, the specific intracellular mechanisms have not been clearly delineated. This cartoon depicts possible scenarios. After diffusion of the protonated SCFA across the cell membrane, there is a rapid dissociation and release of protons. These protons lead to a decrease in pH$_i$, stimulation of Na/H exchange, and inhibition of pH-sensitive K channels. The stimulation of Na/H exchange increases intracellular Na and cell volume. The change in cell volume may lead to the activation of K and Cl channels. The fate of the ionized SCFA (A$^-$) released on dissociation has not been investigated. Because of the difference between pH$_i$ and pK of the SCFAs, one would predict a significant accumulation of A$^-$. If, where, and how A$^-$ exits the cell remains to be determined.
Gradient stimulation of acid-base movement.
Crypt-to-serosa gradients of SCFAs stimulate alkalinization of the crypt lumen and acidification of the subepithelial tissue. Thus, just as a proton gradient may stimulate SCFA absorption (13), a SCFA gradient can drive net proton movement across the epithelium (3, 4). This is not specific for SCFAs; gradients of other weak electrolytes, including CO₂/HCO₃ and ammonia, can also drive acid-base movement across the epithelium (3, 4). The crypt may function as a pH microdomain because of slow mixing with bulk superfusates and contribute significantly to the buffering capacity of the lumen. The movement of acid-base equivalents induced by a SCFA gradient could set in motion the movement of other weak electrolytes and may, in part, explain in vivo “bicarbonate secretion” or ammonia absorption that invariably accompanies SCFA absorption (3, 4).

Recent studies in perfused crypts suggest that there is a significant apical diffusion barrier to SCFAs. Luminal perfusion of SCFAs elicits relatively minor changes in pHᵢ compared with serosal exposure. If, indeed, such a barrier exists, this may explain how colonocytes adapt to high concentrations of SCFAs. However, in other preparations examining the “sidedness” of the effects of SCFAs, a similar luminal barrier to diffusion was not seen (3, 4, 12).

Basic models of volume regulation parallel those of pHᵢ regulation. SCFAs cause cells to swell. There are two probable components to this volume increase. Diffusive entry of protonated SCFA provides a small, initial increase in cell volume followed by a larger increase that is mediated by NHE. Isolated colonic crypts superfused with SCFAs show a differential volume effect: mature surface cells have a larger increase in cell volume than crypt cells (6). This may be caused by the increased number of apical NHEs in mature colonocytes compared with relatively undifferentiated cells at the base of the crypt (13). However, in some colonic epithelia, cell swelling may occur in Na-free solutions or with inhibition of NHE (14). Thus, similar to pHᵢ responses, there may be multiple mechanisms of SCFA-induced cell swelling, some dependent on NHE and others not.

After SCFA-induced swelling, regulatory volume decrease (RVD) is seen under some, but not all, conditions. This variable response appears to be related to methodological differences (11, 15), but the specific transporters involved in RVD remain elusive. Different stimuli for cell swelling may recruit different transporters for reestablishment of normal cell volume. In other words, although both hypotonia and SCFA induce cell swelling, they may involve different homeostatic mechanisms to return the cell towards baseline volumes (10). In large part, RVD is mediated by the opening of volume-sensitive K⁺ and Cl⁻ channels. Diener (6) has identified a specific volume-sensitive Cl⁻ channel regulated by leukotrienes involved in SCFA RVD. In contrast, in HT29 cells, SCFA-induced RVD is mediated by a 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid (SITS)-sensitive, Cl⁻-dependent transporter that is not a channel (11).

SCFA-induced modulation of Na⁺ and Cl⁻ transport
In vivo, SCFAs stimulate Na⁺ absorption. In vitro, the enhanced Na absorption has generally been characterized as electroneutral and linked to Cl⁻ absorption. An initial decrease in pHᵢ, either through diffusion of protonated SCFA or via SCFA/HCO₃ exchange, activates an apical NHE and increases apical Na entry. Na then exits the cell through the basolateral Na pump. The resulting intracellular alkalinization activates an apical Cl⁻/HCO₃ exchange, promoting Cl⁻ absorption. Observations that SCFA-stimulated Na absorption correlates with the presence of apical NHEs (8, 13) support this model. If the critical factor in SCFA stimulation of Na absorption is a decrease in pHᵢ, this then leads to several intriguing questions about the functional responses of apical and basolateral NHEs.

Assuming that both mucosal and serosal SCFAs acidify colonocytes, then, for example, basolateral or bilateral SCFAs should be equally...
effective in stimulating Na\(^+\) absorption as apical SCFAs (Fig. 3). This does not appear to be the case; a mucosal-to-serosal SCFA gradient is a more effective absorptive stimulus than a reversed serosa-to-mucosa gradient.

For Na\(^+\) absorption to occur, there must be selectively greater activation of the apical NHE compared to the basolateral NHE. Theoretically, this may be caused by 1) a greater number of apical versus basolateral NHEs in the absorptive cells; 2) a differential sensitivity to decreases in pH; 3) a pH microdomain created by SCFAs; or 4) a more direct linking of SCFAs to the Na\(^+\)-absorptive mechanism. There does not appear to be any evidence for the first two possibilities. A pH modifier site that activates Na/H exchange at lower pH has been described for NHE-1 (basolateral NHE) but not convincingly for the apical exchanger. This differential sensitivity would, if anything, favor Na\(^+\) secretion.

However, there may be a polarity to the NHE response to SCFAs; mucosal SCFAs may more effectively stimulate apical NHEs (1, 12). There is a precedent for a polarized effect of SCFAs on epithelia: there are significantly different electrophysiological responses when the gallbladder is exposed to either mucosal or serosal propionate. Equivalent degrees of intracellular acidification in rat colonic epithelia do not have the same stimulatory effect on Na absorption. This has led Charney and Dagher (1) to hypothesize that there is a subapical pH microdomain sensitive to SCFAs (and CO\(_2\)) that may regulate Na absorption stimulated by weak electrolites.

Under some circumstances, SCFAs can stimulate Na\(^+\) absorption without increasing Cl absorption, while still maintaining electroneutrality (5). This indicates a corresponding increase in the movement of another ion. One possibility is that SCFAs may share common Na-linked transport mechanisms, e.g., Na-HCO\(_3\)-SCFA symport. Early observations in gallbladder pointed out that SCFAs and bicarbonate were relatively interchangeable in stimulating electroneutral Na absorption. Early models posited a Cl/HCO\(_3\)/SCFA apical exchanger, but detailed vesicle studies have raised questions about the presence of such an antiporter. SCFAs could “substitute” for HCO\(_3\) by indirectly promoting an increase in cellular metabolism and intracellular production of HCO\(_3\). If this were the case, then a poorly metabolized SCFA (isobutyrate) would have a smaller effect than an efficiently metabolized SCFA such as butyrate. Although this has occasionally been seen, most investigators have not found this to be the case. Although SCFAs have been linked to Cl transport, recent observations have suggested a regulatory role in electrogenic Cl secretion. SCFAs, especially butyrate, inhibit cAMP-stimulated Cl secretion independent of the effects on absorptive fluxes. This effect is at the apical membrane, but the specific mechanisms remain to be defined (1).

Studies on the effects of SCFAs on colon provide an opportunity to apply many of the recent advances in the molecular biology and electrophysiology of transport. The mechanism(s) for SCFA entry and exit from the colonocyte are not entirely resolved, nor do we fully understand SCFA-induced volume and pH regulation. Characterizing the interrelationships among SCFAs, pH\(_r\), and the family of NHEs in colonocytes provides an opportunity to define the physiological regulation of NHEs. Determining which effects are specific for SCFAs and which are more generalized actions of weak electrolites is an important and necessary step in sorting out the physiological (and pathophysiological) roles for SCFAs. Because there are major alterations in luminal SCFAs in several colonic diseases and SCFAs may have a therapeutic role in treating a variety of these diseases, the answers to these questions may have significant clinical importance.

References


15. Sellin, J., and H. Shelat. Short-chain fatty acid (SCFA) vol-