J. Membrane Biol. 150, 83–88 (1996)

The Journal of
Membrane
Biology

Short-Chain Fatty Acid (SCFA) Volume Regulation in Proximal and Distal Rabbit Colon is Different

J. Sellin, H. Shelat
Division of Gastroenterology, Dept. of Internal Medicine, The University of Texas Medical School Houston, Texas

Received: 1 September 1995/Revised: 9 November 1995

Abstract. SCFAs increase the volume of many different cell types rarely exposed to significant concentrations of these weak electrolytes. SCFAs swell isolated cells from colonic carcinoma cell lines, but the mechanism(s) of volume regulation in normal colonocytes, which are generally exposed to >100 mM SCFAs, has not been well characterized. Aims: To determine the effect of SCFAs on volume regulation in proximal and distal rabbit colonocytes. Methods: Isolated colonocytes were plated on coverslips and placed in a perfusion apparatus that permitted fluid changes. Cells were continuously monitored by video-microscopy; volume was estimated by measured changes in the radius of individual cells. Results: Distal colonocytes (DC) consistently had a slightly greater basal volume than proximal colonocytes (PC): [14.2 pl/fl:9.8 pl/fl] In HEPES-buffered solutions, an isotonic change to a 90 mM NaCl/50 mM Na propionate solution elicited a significant increase in cell volume within 10 min, but no noticeable regulatory volume decrease over 30 min: V/Vo in DC: 1.29 ± .09; in PC: 1.25 ± .05. In HCO_3-buffered solutions, 50 mM PROP caused significantly greater cell swelling; in DC: 1.74 ± .21; in PC: 1.52 ± .08. In DC both amiloride and EIPA blocked the SCFA-induced increase in cell volume. A hypotonic challenge confirmed that these cells were capable of swelling. In contrast, amiloride did not significantly inhibit SCFA-induced swelling in PC: control, 1.25 ± .05; amiloride, 1.36 ± .10. Cell volume increased in PC perfused with an isosmotic 50 mM propionate, Na-free solution: 1.22 ± .04. Conclusions: (i) SCFAs induce significant cell swelling, but no regulatory volume decrease, in isolated colonocytes; (ii) HCO_3 augments SCFA-induced cell swelling; (iii) volume increase in DC is dependent on Na-H exchange, but in PC appears to be Na-independent. Significance: There are fundamental differences in how proximal and distal colon respond to isosmotic volume challenge of SCFAs.

Key words: Short-chain fatty acids — Colon — Na-H exchange — Amiloride — Bicarbonate

Introduction

An intricate relationship exists between contents of the colonic lumen and the function of epithelial cells. In addition to transport either into or out of the colonic lumen, epithelial cell functions such as regulation of intracellular pH and maintenance of cell volume may be modulated by specific components of the luminal fluid. Short-chain fatty acids (SCFAs), the predominant luminal anions in colonic luminal fluid, are a prime candidate for having such an effect. Previous studies, from our laboratory and others', have shown that SCFAs are readily absorbed across the colonic epithelium [5, 14–16] and acidify the intracellular pH of colonocytes [3].

Maintenance of cell volume within a critical range is a vital homeostatic function of all cells. Regulatory volume responses to osmolar challenges have been well-described [9]. Within the gut lumen, major variations from isosmolar conditions are limited, restricted to the stomach, upper small intestine and cecum. In addition to osmolar challenges, cells necessarily require volume-regulatory responses in situations when there is no significant changes in osmolarity, but when the external milieu contains a significant proportion of freely diffusible substances, most commonly weak electrolytes. This process has been termed isosmotic volume regulation in contrast to the response to hyper- or hypotonic conditions [9]. Such processes are particularly germane to the colonic epithelium, which is exposed to high luminal concentrations of weak electrolytes, specifically short-

Correspondence to: J.H. Sellin

chain fatty acids (SCFAs), in the absence of a large osmotic gradient.

Isosmotic cell swelling induced by SCFAs has been postulated to occur by two mechanisms: (i) an initial diffusion of protonated, neutral SCFAs into cells causing both an increase in intracellular osmolarity and a decrease in intracellular pH (pH_i) following dissociation of the SCFA, and (ii) stimulation of Na-H exchange mechanisms, secondary to intracellular acidification, leading to an increase in intracellular Na.

In this study, we sought to examine the volume responses of isolated colonocytes to physiologic concentrations of SCFAs; employing a video-microscopy system to provide continuous monitoring of individual isolated cells, we found major regional differences between proximal and distal colonocytes to a SCFA challenge. Although distal colonocyte swelling is dependent on Na-H exchange, proximal colonocyte increase in cell volume appears to be Na-independent.

Materials and Methods

Cell Isolation

New Zealand white male rabbits (2–3 kg) were fed standard rabbit chow and water ad libitum. Rabbits were sacrificed by ear vein injection with Euthanasia solution (Beuthanasia-D, Schering-Plough Animal Health, Kenilworth, NJ) following protocols approved by institutional animal welfare committee. The appropriate segment of colon was removed rapidly, opened along its mesenteric border and rinsed of luminal contents. The serosa and muscle were removed by peeling off the layers with a glass slide. The mucosal layer was fixed on a plastic holder with tissue adhesive cyanoacrylate ester (SuperGlue, distributed by Tepco Associates, Skokie, IL) and transferred to a Parson's EDTA solution for 30 min at 37°C. The mucosa attached to the plastic holder was vibrated once for 30 sec to isolate mostly individual cells, primarily surface cells, which were collected and stored in an intracellularlike high K Tyrode buffer. Cell viability with this method provides a >90% viability [3].

Morphometry

Cells were fixed to poly-1-lysine-coated coverslips following centrifugation using a Cytospin-3 (Shandon Instruments, Pittsburgh, PA). The perfusion chamber (RC21B, Warner Instruments, Hamden, CT) provided high optical resolution and a small reservoir volume (0.3 ml) permitting perfusion rates of approximately 1 ml/min. Solutions were maintained at 37°C. The video monitoring system has been described in detail previously [11]. Briefly, the chamber was mounted on a Nikon inverted microscope interfaced with an MTI series 68 video camera (Dage-MTI, Michigan City, IN) attached to a VCR and video monitor, linked to a video timer (For-A Co, Japan) and IV-530 contour synthesizer. The cell images were taken on high quality thermal paper with a video graphic printer (Sony UP-811N).

The diameter of individual colonocytes was measured using a calibrated ocular at 3400×. The focal plane for cells selected for measurement was adjusted to provide for maximal cell diameter through the cell center. Images were recorded every minute. The diameter of individual cells was measured at 4–6 axes; volume was calculated using standard formulae.

A similar protocol was used for all experiments. A single round cell was identified and observed for 5 min to establish a baseline volume (V_o). Change in perfusate at time 0 marked the specific experimental intervention; the individual cell was then observed for 25–30 min. At the end of each protocol, cells were perfused with hypotonic media to ascertain cell responsiveness, analogous to the glucose challenge at the end of an Ussing chamber flux experiment. Cells reliably swelled in response to the hypotonic challenge and returned towards baseline volume with a switch back to isotonic perfusate. (*Data not shown.*) Results are expressed in two different ways: (i) V_{max}/V_o, the ratio of the maximal volume the cell reaches compared to the initial volume, or (ii) a time course of a group of cells over the duration of the perfusion. Because different cells did not reach V_{max} at an identical time during a perfusion, the V_{max}/V_o data generally show a greater increase in volume than the time course.

Solutions

The EDTA solution for cell isolation contained (in mmol/l) NaCl 107, KCl 4.5, $NaHPO_4$ 0.2, Na_2HPO_4 1.8, $NaHCO_3$ 25, EDTA (ethylenedinitrilotetraacetic acid) 10, glucose 10, with 0.1% bovine serum albumin. pH was adjusted to 7.4 with TRIS. The high K Tyrode solution contained K gluconate 100, KCl 20, NaCl 20, $CaCl_2$ 1.25, $MgCl_2$ 1.0, HEPES (N-(2-hydroxyethyl)piperazine-N-2-ethanesulphonic acid) 10, glucose 10, sodium pyruvate 5, with 0.1% bovine serum albumin. Experiments were carried out in either a HEPES or bicarbonate buffered Ringer solution gassed with 100% O_2 or 95% O_2/5% CO_2. In most solutions, Na-SCFAs were substituted on an equimolar basis for NaCl. In the Na-free solutions, N-methyl-D-glucamine was substituted for Na.

Statistics

Statistical significance between groups was determined by unpaired *t*-tests.

Results

Initial Volumes

The resting volume of colonocytes varied depending on both the location and the bathing solution. In HEPES-buffered solutions, isolated cells from the distal colon were larger than those from proximal. (15.8 ± 1.1 ($n = 49$) *vs.* 10.6 ± 1.0 ($n = 15$) fl, $P < 0.01$) In HCO_3-buffered solutions, cells from each site were smaller, but the differential between distal and proximal colon remained. (11.9 ± 0.5 ($n = 17$) *vs.* 8.6 ± 0.4 ($n = 16$), $P < 0.01$). Removal of Na (N-methyl-*D*-glucamine substitution) did not significantly change the volume of proximal colonocytes (9.8 ± 1.3 ($n = 8$) *vs.* 10.6 ± 1.0, ($n = 15$)). Thus distal colonocytes were consistently larger than proximal colonocytes independent of buffer. Cells exposed to bicarbonate were smaller than those bathed in a HEPES buffer.

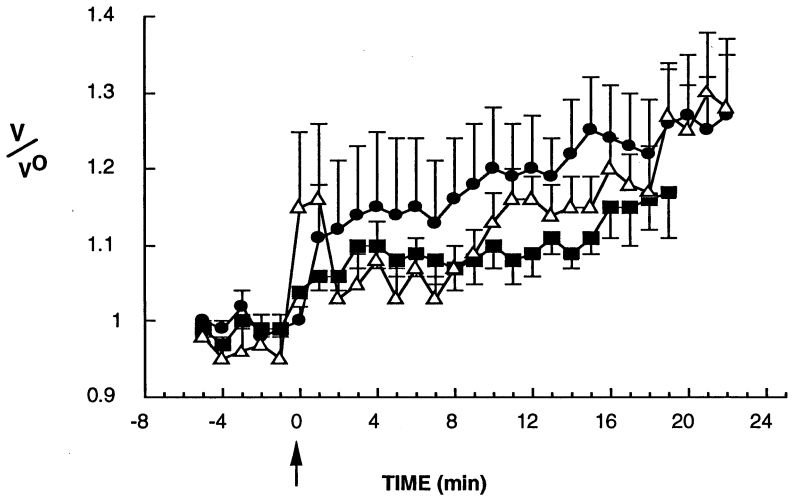

Fig. 1. Time course of volume changes in proximal colon. The cell volume of isolated colonocytes increases with changing perfusate from a HEPES-buffered saline to solutions containing (in mM): 90 NaCl, 50 Na propionate in HEPES (■–■–■–), 90 NaCl, 50 Na propionate in a HCO_3 buffer (●–●–●), and 140 Na propionate in a HEPES buffer (△–△–△). Perfusate change at time 0. V/V_o represents the ratio of volume changes over time compared to initial volume. Fifty mM PROP/HEPES significantly increases volume over baseline; a change from HEPES to HCO_3, but not an increase to 140 mM PROP, caused a significantly greater increase in volume compared to 50 mM propionate in HEPES buffer. (*See text.*)

RESPONSE TO SCFAS

Time Course

At time 0, we changed the perfusate from 140 mM NaCl to 90 mM NaCl/50 mM propionate. This elicited an increase in cell volume over the ensuing 20 min in both proximal and distal colon; we did not observe a predictable regulatory volume decrease during the period of observation. (Figs. 1 and 2)

Effect of Varying Buffers

Varying the principal buffer from HEPES to HCO_3 altered the response to a SCFA challenge. In the presence of bicarbonate, 50 mM propionate elicited a greater volume increase in both proximal and distal colon. In proximal colon, V_{max}/V_o in HEPES was $1.3 \pm .1$ ($n = 10$); with HCO_3 buffering, the ratio was significantly increased ($1.5 \pm .1$ ($n = 11$), $P < 0.05$). In distal colon, V_{max}/V_o was $1.3 \pm .1$ ($n = 9$); with HCO_3 buffering, the ration increased to $1.7 \pm .2$ ($n = 11$), ($P < 0.01$). This suggests that the presence of external HCO_3 alters volume regulatory mechanisms, most likely by either facilitating propionate entry or slowing propionate exit from colonocytes.

Additionally, we tested whether changes in SCFA chain length or concentration would alter the effect on cell volume. In proximal colon 140 mM propionate-HEPES increased cell volume; however, the increase in V_{max}/V_o (1.4 ± 0.1) induced by 140 mM propionate was not significantly greater than that of 50 mM propionate-HEPES. Increasing chain length increases the lipid solubility of SCFAs and should increase diffusive entry of SCFAs into colonocytes. The 4-carbon SCFA butyrate (50 mM) in HEPES elicited a V_{max}/V_o of $1.4 \pm .1$ in distal colon; the butyrate-induced swelling was similar to that stimulated by an equimolar propionate challenge.

Regulation by [Na] and Na-H Exchange

Previous studies have shown that the Na-H exchanger is integral to the increase in volume induced by SCFAs in either transformed cell lines or isolated rat distal colon crypts. Therefore, we examined how the Na-H exchange inhibitor amiloride modulated the increase in volume induced by 50 mM propionate (Fig. 3). Amiloride alone had no significant effect on cell volume. In distal colon, 10^{-3} M amiloride significantly inhibited the propionate-associated increase in cell volume. Amiloride also inhibited the volume increase elicited by butyrate. To rule out that this inhibition was due to an unanticipated effect of amiloride on other transporters, e.g., Na channels, we repeated these studies employing the more specific Na-H exchange inhibitor, EIPA. EIPA had essentially the same effect as amiloride. These cells were quite capable of increasing cell volume. A hypotonic challenge in the presence of EIPA elicited a rapid and significant increase in cell volume: V_{max}/V_o $1.5 \pm .2$.

The response in proximal colonocytes was very different. Amiloride failed to block the increase in volume by either 50 or 140 mM propionate. To more specifically examine the role of [Na], we examined the effect of entirely removing [Na] from the perfusate. In an NMDG-Ringer, proximal colonocytes increased cell volume in response to 50 mM propionate. The combination of the Na-free and the amiloride data suggest that propionate-induced cell swelling in the proximal colon is fundamentally different from that in the distal colon and not directly dependent on [Na].

Discussion

This study demonstrates that physiologic concentrations of SCFAs increase cell volumes in isolated epithelial

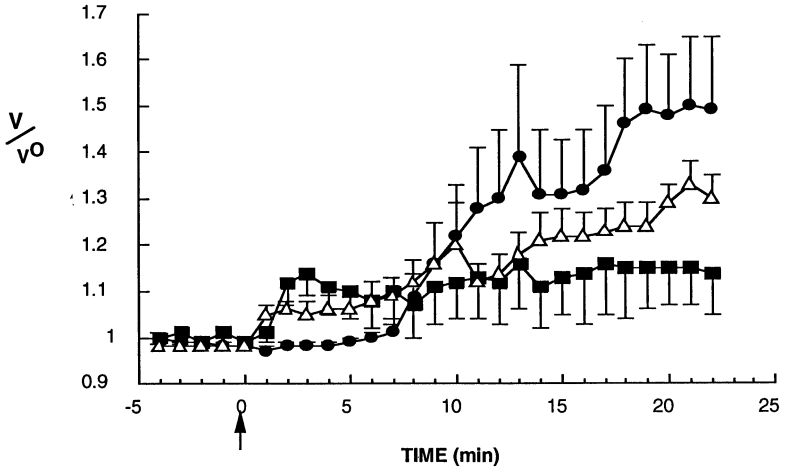

Fig. 2. Time course of volume changes in distal colon. The cell volume of isolated colonocytes increases with changing perfusate from a HEPES-buffered saline to solutions containing (in mM): 90 NaCl, 50 Na propionate in HEPES (■–■–■–), 90 NaCl, 50 Na propionate in a HCO_3 buffer (●–●–●), and 50 Na butyrate in a HEPES buffer (△–△–△). Perfusate change at time 0. V/V_o represents the ratio of volume changes over time compared to initial volume. Fifty mM PROP/HEPES significantly increases volume over baseline; a change from HEPES to HCO_3, but not to butyrate, caused a significantly greater increase in volume compared to 50 mM propionate in HEPES buffer. (*See text.*)

cells from both the proximal and distal colon. As with ion transport, there are major segmental differences in volume regulation along the colon. SCFA-induced swelling is dependent on Na-H exchange in distal colon, but does not appear to be Na-dependent in proximal colon. Both proximal and distal colon exhibit greater increases in volume in HCO_3 compared to HEPES-buffered solutions.

A limited number of previous studies have examined the issue of SCFA-induced volume regulation in colonic epithelia [4, 6, 12]. They have all found that SCFAs induce cell swelling, but there have been important differences in methodology and results. It is difficult to readily compare data between different studies because of differences in cell type and methods; the most relevant studies have been done in HT29 cells in suspension (electronic sizing) [12], shark rectal gland (water content) [6], isolated crypts from rat distal colon (crypt diameter) [4], and isolated colonocytes from proximal and distal rabbit colon adherent to coverslips (*present study*).

One of the differences among studies is the presence of a regulatory volume decrease (RVD) in the continued presence of SCFAs. Rowe et al., observed a 30% RVD, only in the presence of Cl, in HT-29 colonic carcinoma cell line measured by electronic sizing in HEPES buffer [12]. Diener et al., found a slight but significant RVD in HCO_3-bathed, but not HEPES-bathed, isolated rat colonic crypts [4]. Feldman observed no RVD in shark rectal glands [6]. We did not detect RVD in isolated colonocytes in either HEPES or HCO_3 buffers. Thus, RVD is not a consistent finding after isosmotic SCFA-induced swelling. Although the potential mechanisms of RVD (or the lack) may be of considerable import, we will restrict our discussion to the issue of cell swelling as found in our experimental model.

Effects of HCO_3 on Basal and Stimulated Volumes

Changes in buffer (HEPES *vs.* HCO_3) changed both the resting volumes and the magnitude of the volume response to SCFAs. To our knowledge, this is the first study that examined the effects of alterations of buffer on resting colonocyte cell volumes. HCO_3, as a permeable weak electrolyte similar to SCFAs, may have been expected to increase basal cell volume. HCO_3 may significantly alter resting pH_i; if it caused acidification, one might expect inhibition of pH-sensitive channels and an increase in basal cell volume [1, 8]. Neither of these mechanisms appears to be operative in isolated colonocytes, in which HCO_3 is associated with decreased volumes. Alternatively, Dagher has demonstrated that bicarbonate, rather than pH, may alter basal rates of Cl transport in rat distal colon [2]. This suggests that HCO_3 may modulate a transport system that affects basal cell volume. Theoretically, HCO_3 may be expected to increase cell volume by (i) functioning as a permeant weak electrolyte or (ii) decreasing basal pH_i with subsequent inhibition of pH-sensitive K channels [1, 8].

The presence of HCO_3 enhances the SCFA-induced volume response in isolated colonocytes. Diener et al., proposed that a bicarbonate-containing solution, by providing a more effective intracellular buffer, decreases SCFA-induced acidification and therefore dampens pH_i driven Na entry through NaH exchangers [4]. We have previously shown that, in isolated proximal colonocytes, substitution of a bicarbonate for a HEPES buffer, lessens the acidifying effects of propionate [3]. Therefore, increased cell swelling in the HCO_3-containing solutions cannot be ascribed to enhanced Na-H exchange activity secondary to a greater decrease in pH_i. In fact, we would expect a smaller decrease in pH_i. Instead, HCO_3 may be altering other transporters, perhaps by either facilitating an entry or inhibiting an exit step for SCFAs in these cells.

Na-independent Volume Increases in Proximal Colon

The alterations in volume regulation in response to inhibition of Na-H exchange in distal colon is consistent with previous models implicating two factors in SCFA-

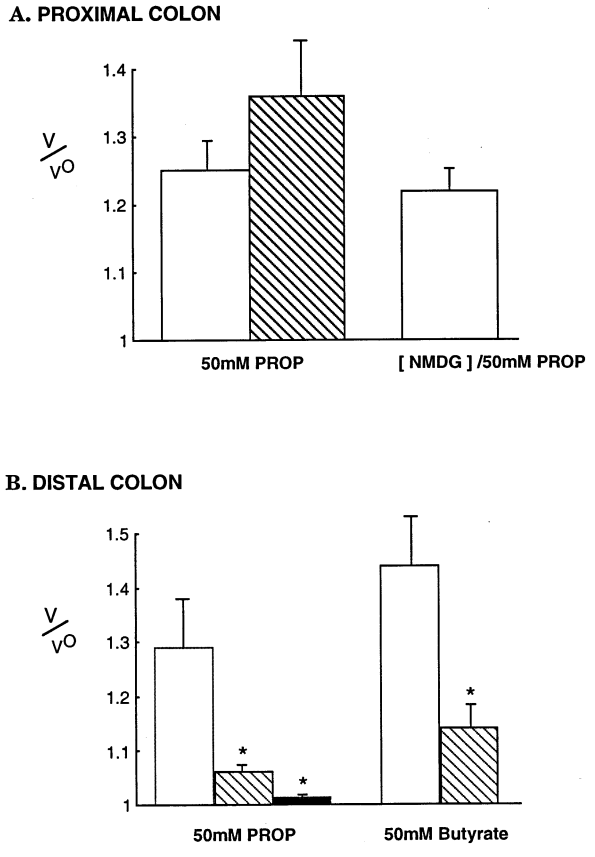

Fig. 3. Differential effects of Na and Na-H exchange inhibition in proximal and distal colon. Amiloride (10^{-3} M) blocks the SCFA-induced increase in volume in distal, but not proximal, colon. EIPA (10^{-6} M), an amiloride analogue specific for Na-H exchange, has a similar effect in distal colon. In proximal colon, the effect is independent of Na as well as Na-H exchange. *$P < 0.05$ *vs.* control. (□) Control; (▨) amiloride; (■) EIPA.

induced cell swelling: (i) an initial diffusion of the protonated weak acid, and (ii) a subsequent increase in cell Na in response to stimulated Na-H exchange. The inhibition of the volume increase by the amiloride analogues is consistent with a pivotal role for Na-H exchange. The residual swelling in the presence of amiloride (butyrate > propionate) suggests that diffusive entry is important.

However, several findings suggest that there may be considerably more complexity to the mechanisms of increases in cell volume. Acidification of the cell interior by mechanisms other than SCFAs does not ineluctably lead to an increase in cell volume [7]. Given that the basolateral Na pump is generally accepted to have the capacity to respond to increases in Na entry into the cell (homocellular regulation) [13], it unclear why, in the case of SCFA-induced volume increases, Na extrusion via the pump does not keep up with accelerated Na-H exchange. For example, nutrient-induced increases in cell volume are generally short-lived and the cells return to baseline volumes rapidly (<5 min) [10]. The role of intracellular SCFA anions released by dissociation of the protonated acid has not been investigated and may play an important role in the volume increases.

Finally, our finding of SCFA-induced swelling in PC independent of Na or Na-H exchange contrasts with our results in distal colon and those of other investigators [4, 6, 12]. The observed increase in volume suggests that significant differences in membrane permeabilities, ion transporters, and pH_i regulation exist between different colonic segments.

SCFA-induced changes in pH_i can be used as surrogates for cellular uptake and exit of SCFAs. In this regard, we have previously demonstrated that isolated proximal colonocytes in Na-free media respond to a propionate challenge by acidification and recovery of pH_i in a similar manner to cells in Na-containing media [3]. Such acidification demonstrates Na-independent entry of SCFAs into colonocytes. The normalization of pH_i suggests that (i) a Na-independent acid extrusion mechanism may be operative and, (ii) a driving force for continued SCFA entry can be maintained in the absence of Na. Thus, proximal colonocytes exhibit both Na-independent volume and pH_i regulatory capacity. The mechanisms for these responses remain to be determined.

In summary, these data demonstrate that SCFAs elicit predictable volume increases in isolated rabbit colonocytes. The characteristics of volume increase vary depending on colonic segment (proximal *vs.* distal), buffer (HCO_3 *vs.* HEPES) and the effect of Na and Na-H exchangers. Future studies measuring volume and pH_i simultaneously will permit further exploration of the mechanisms involved in these responses.

We would like to acknowledge the assistance of Dr. Roger O'Neil in the Department of Integrative Biology at The University of Texas Medical School-Houston for his use of the equipment used in this study and the Crohn's & Colitis Foundation of America for their generous support which made this study possible.

References

1. Copello, J., Segal, Y., Reuss, L. 1991. Cytosolic pH regulates Maxi K channels in *Necturus* gallbladder epithelial cells. *J. Physiol.* **434:**577–590
2. Dagher, P.C., Balsam, L., Weber, J.T., Egnor, R.W., Charney, A.N. 1992. Modulation of chloride secretion in the rat colon by intracellular bicarbonate. *Gastro.* **103:**120–127
3. DeSoignie, R., Sellin, J.H. 1994. Propionate-initiated changes in intracellular pH in rabbit colonocytes. *Gastro.* **107:347–356**
4. Diener, M., Helmle-Kolb, C., Murer, H., Scharrer, E. 1993. Effect of short-chain fatty acids on cell volume and intracellular pH in rat distal colon. *Pfluegers Arch.* **424:**216–223
5. Engelhardt, W.V., Rechkemmer, G. 1992. Segmental differences of short-chain fatty acid transport across guinea-pig large intestine. *Exp. Physiol.* **77:**491–499
6. Feldman, G.M., Ziyadeh, F.N., Mills, J.W., Booz, G.W., Kleinzeller, A. 1989. Propionate induces cell swelling and K^+ accumulation in shark rectal gland. *Am. J. Physiol. Cell Physiol.* **257:**c377–c384
7. Hanrahan, J.W., Tabcharani, J.A. 1990. Inhibition of an outwardly

rectifying anion channel by HEPES and related buffers. *J. Membrane Biol.* **116:**65–77

8. Klaerke, D.A., Wiener, H., Zeuthen, T., Jorgensen, P.L. 1993. Ca^{2+} activation and pH dependence of a maxi K^+ channel from rabbit distal colon epithelium. *J. Membrane Biol.* **136:**9–21
9. Lewis, S.A., Donaldson, P. 1990. Ion Channels and Cell Volume Regulation: Chaos in an Organized System. *NIPS* **5:**112–119
10. MacLeod, R.J., Hamilton, J.R. 1991. Volume regulation initiated by Na-nutrient cotransport in isolated mammalian villus enterocytes. *Am. J. Physiol.* **260:**G26–G33
11. McCarty, N.A., O'Neil, R.G. 1990. Dihydropyridine-sensitive cell volume regulation in proximal tubule: the calcium window. *Am. J. Physiol.* **259:**F950–F960
12. Rowe, W.A., Blackmon, D.L., Montrose, M. 1993. Propionate activates multiple ion transport mechanisms in the HT29-18-Cl human colon cell line. *Am. J. Physiol.* **265:**G564–G571
13. Schultz, S.G., Hudson, R.L. 1991. Biology of sodium-absorbing epithelial cells: dawning of a new era. *In:* Handbook of Physiology—The Gastrointestinal System IV, S.G. Schultz, editor, pp. 45–81. American Physiology Society, Bethesda
14. Sellin, J.H., DeSoignie, R. 1990. Short-chain fatty acid absorption in rabbit colon in vitro. *Gastro.* **99:**676–683
15. Sellin, J.H., DeSoignie, R., Burlingame, S. 1993. Segmental differences in Short-Chain Fatty Acid Transport in Rabbit Colon: Effect of pH and Na. *J. Membrane Biol.* **136:**147–158
16. V. Engelhardt, W., Burmester, M., Hansen, K., Becker, G., Rechkemmer, G. 1993. Effects of amiloride and ouabain on short-chain fatty acid transport in guinea-pig large intestine. *J. Physiol.* **460:**455–466