

# Effects of butyrate on active sodium and chloride transport in rat and rabbit distal colon

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Short chain fatty acids, particularly butyrate, stimulate electroneutral NaCl absorption from the colon. Their effect in colonic epithelia lacking basal electroneutral NaCl absorption is unknown. Butyrate is also reported to inhibit active Cl<sup>-</sup> secretion in the colon. The present studies were undertaken to investigate the inter-relationships between the effects of butyrate on active Na<sup>+</sup> and Cl<sup>-</sup> transport in the colon. Studies were carried out in rabbit distal colon (known to have predominant electrogenic Na<sup>+</sup> absorption), rat distal colon (characterised by electroneutral Na<sup>+</sup> absorption), and hyperaldosteronaemic rat distal colon (characterised by electrogenic Na<sup>+</sup> absorption). The effect of cholera toxin (CT) was also noted. Potential difference, short-circuit current ( $I_{sc}$ ) and fluxes of Na<sup>+</sup> and Cl<sup>-</sup> were measured in stripped mucosa under voltage-clamp conditions. Butyrate stimulated electroneutral Na<sup>+</sup> and Cl<sup>-</sup> absorption in distal colon of normal and salt-depleted rats, and stimulated Na<sup>+</sup> absorption in rabbit distal colon. Amiloride (10<sup>-4</sup> M) or CT did not inhibit this process. In rabbit distal colon, stimulation of Na<sup>+</sup> absorption by butyrate was not dependent on the presence of Cl<sup>-</sup> in the medium. Butyrate significantly decreased conductance, decreased flux of sodium from serosa to mucosa (particularly in rabbit distal colon), and decreased  $I_{sc}$ . Net Cl<sup>-</sup> secretion, induced by CT, was completely inhibited by butyrate. Stimulation of Na<sup>+</sup> absorption was independent of exposure to CT. Bumetanide reversed net Cl<sup>-</sup> secretion to net absorption, but did not alter Na<sup>+</sup> or Cl<sup>-</sup> fluxes in tissues exposed to butyrate. Thus butyrate stimulates active Na<sup>+</sup> absorption in colonic epithelia, with or without expression of basal Na<sup>+</sup>-H<sup>+</sup> exchange. Independently, butyrate inhibits active Cl<sup>-</sup> secretion induced by cAMP in these epithelia.

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Short chain fatty acids (SCFA), products of bacterial fermentation of unabsorbed carbohydrate, are the major anions of the colon in health. The important SCFA in the normal human colon are acetate, propionate and butyrate, which together reach or exceed concentrations of 100 mM. Of these, acetate is the most abundant, but butyrate plays the most important role in colonic physiology. SCFA play an important role in modulating colonic electrolyte transport. The ability of SCFA to stimulate sodium absorption from the mammalian colon has been shown *in vivo* and *in vitro* (Bugaut, 1987). Flux studies *in vitro* have shown that SCFA stimulate an electroneutral Na<sup>+</sup> and Cl<sup>-</sup> absorptive process in the colon (Binder & Mehta, 1989). The SCFA-linked absorption pathway appears to involve the movement of SCFA across the apical membrane into the colonic epithelial cell, through either non-ionic diffusion or SCFA-HCO<sub>3</sub><sup>-</sup> exchange, followed by stimulation of Na<sup>+</sup>-H<sup>+</sup>, Cl<sup>-</sup>-SCFA and Cl<sup>-</sup>-HCO<sub>3</sub><sup>-</sup> exchanges across the apical membrane of the colon (Rajendran & Binder, 1994; Charney *et al.* 1998). SCFA-dependent electroneutral NaCl transport is distinct from bicarbonate-dependent electroneutral NaCl transport, and is now established as one of the major transport

pathways for Na<sup>+</sup> in the mammalian colon. Cyclic AMP (cAMP) causes net secretion by inhibiting bicarbonate-dependent electroneutral NaCl absorption in surface epithelial cells and by turning on electrogenic Cl<sup>-</sup> secretion in crypt epithelial cells. *In vivo* studies have shown that butyrate inhibits net fluid secretion induced by cholera toxin in the rat colon (Ramakrishna *et al.* 1990). In studies using rat distal colon mounted in flux chambers *in vitro*, theophylline failed to inhibit butyrate-linked NaCl absorption (Binder & Mehta, 1990), suggesting that butyrate-dependent NaCl absorption was not sensitive to the effect of cAMP. On the other hand, studies using rat distal colon mucosa *in vitro* have shown that butyrate had an inhibitory effect on cAMP-induced Cl<sup>-</sup> secretion, which is the process underlying toxin-induced fluid secretion (Dagher *et al.* 1996).

Electrogenic Cl<sup>-</sup> secretion, via chloride channels in the apical membrane of epithelial cells, is the fundamental means by which mucosal surfaces are hydrated in health. Defective regulation of this process underlies a number of diseases of considerable importance, including secretory diarrhoea and cystic fibrosis. Multiple intracellular pathways

acting through cAMP and cGMP, calcium/calmodulin and diacylglycerol regulate these chloride channels (Berger *et al.* 1993; Gabriel *et al.* 1993; Vaandrager *et al.* 1998; Seibert *et al.* 1999). Disturbance in one or more regulatory pathways leads to hypersecretion in the intestine, causing secretory diarrhoea. For electrogenic  $\text{Cl}^-$  secretion to take place at the apical membrane, chloride has to be internalised into the cell across the basolateral membrane primarily via bumetanide-inhibitable  $\text{Na}^+-\text{K}^+-2\text{Cl}^-$  cotransport. In cAMP-elicited  $\text{Cl}^-$  secretion, activation of apical  $\text{Cl}^-$  channels is generally viewed as the primary regulatory event. However, basolateral  $\text{Na}^+-\text{K}^+-2\text{Cl}^-$  cotransport must also increase to maintain cell electrolyte composition, and therefore active  $\text{Cl}^-$  secretion demands co-ordinated control of apical  $\text{Cl}^-$  exit and basolateral  $\text{Cl}^-$  entry. It has been shown that  $\text{Na}^+-\text{K}^+-2\text{Cl}^-$  cotransport is stimulated by an increase in cytosolic cAMP, and inhibited by divalent cations and by a decrease in ATP levels (Haas, 1989; Hecht & Koutsouris, 1999; Marunaka *et al.* 1999). The factors responsible for 'cross-talk' between apical and basolateral transport events are incompletely defined, and also the complex interaction of various transport elements with the intracellular mediators and secretagogues makes it difficult to clearly identify a specific effect of SCFA on secretion.

$\text{Na}^+-\text{H}^+$  exchange in the apical membrane of colonic epithelial cells is considered essential to the stimulation of  $\text{Na}^+$  absorption by SCFA (Rajendran & Binder, 1994; Gonda *et al.* 1999).  $\text{Na}^+-\text{H}^+$  exchange is present in epithelia that demonstrate electroneutral  $\text{Na}^+$  absorption (e.g. rat distal colon), whereas it is absent in epithelia without electroneutral  $\text{Na}^+$  absorption (e.g. rabbit distal colon). In the latter,  $\text{Na}^+$  absorption across the epithelium is a two-step, electrogenic process involving mucosal amiloride-sensitive  $\text{Na}^+$  channels and active transport across the basolateral membrane via the  $\text{Na}^+-\text{K}^+-\text{ATPase}$  (McCabe *et al.* 1982). The rate-limiting step in this process is apical amiloride-sensitive  $\text{Na}^+$  entry (Thompson & Sellin, 1986; Turnheim *et al.* 1987). The epithelial  $\text{Na}^+$  channel (ENaC) complex is composed of three homologous subunits,  $\alpha$ ,  $\beta$  and  $\gamma$  (McDonald *et al.* 1994; Prince & Welsh, 1998; Stokes & Sigmund, 1998). Aldosterone induces electrogenic  $\text{Na}^+$  absorption in the distal colon, by increasing  $\beta$ - and  $\gamma$ -ENaC mRNA, with little or no effect on  $\alpha$ -ENaC mRNA (Epple *et al.* 2000).

The present studies were performed to determine the effect of butyrate on active  $\text{Na}^+$  and  $\text{Cl}^-$  transport in the colon, i.e. to determine whether it causes stimulation of  $\text{Na}^+$  absorption or inhibition of  $\text{Cl}^-$  secretion, or both. The studies were also designed to determine whether SCFA-dependent  $\text{Na}^+$  absorption would occur in epithelia where electrogenic  $\text{Na}^+$  absorption was the major pathway of  $\text{Na}^+$  absorption, i.e. rabbit distal colon and salt-depleted rats. Salt-depleted rats exhibit suppression of electroneutral  $\text{Na}^+$  absorption with occurrence of electrogenic  $\text{Na}^+$

absorption. This process, i.e. secondary hyperaldosteronism, complicates acute gastroenteritis, resulting in altered transport characteristics of the colon (Rubens & Lambert, 1972), and was therefore included for study. Studies of the butyrate effect on  $\text{Na}^+$  and  $\text{Cl}^-$  transport were therefore carried out in these various epithelia, and the effect on active  $\text{Cl}^-$  secretion induced by cholera toxin was also examined.

## METHODS

### Rat distal colon

Non-fasting male Wistar rats weighing 200–250 g were used for these experiments. Absorption of sodium from rat distal colon is electroneutral in the basal state, and due to  $\text{Na}^+-\text{H}^+$  exchange coupled to  $\text{Cl}^--\text{HCO}_3^-$  exchange (Rajendran & Binder, 1993). Salt depletion on the other hand induces electrogenic amiloride-sensitive  $\text{Na}^+$  transport in the colonic segment with inhibition of electroneutral  $\text{NaCl}$  absorption (Foster *et al.* 1983; Perrone *et al.* 1984; Halevy *et al.* 1986; Sandle & Binder, 1987; Fromm *et al.* 1993; Stokes & Sigmund, 1998; Grotjohann *et al.* 1999). Two groups of animals, a control and a salt-depleted group, were studied. Rats in both groups were fed purified diets based on AIN-93M diet for maintenance of rodents (Reeves, 1997). Normal rats received 500 mg of sodium per kilogram of diet, as recommended by the American National Research Council (Reeves, 1997), while salt-depleted rats received approximately 250 mg sodium per kilogram of diet for 14 days. Plasma aldosterone levels were determined by a commercial radioimmunoassay (Coat-A-Count Aldosterone, DPC, USA), using  $^{125}\text{I}$ -labelled aldosterone antibody-coated tube technology for final separation of free from bound aldosterone. Levels were measured in control and dietary sodium-depleted rat serum at the time of death. The plasma aldosterone level was  $12.4 \pm 3.15$  ng (dl serum) $^{-1}$  in control and  $652 \pm 51.93$  ng (dl serum) $^{-1}$  in salt-depleted rats. Preliminary studies revealed that this serum concentration of aldosterone was reached within 8–10 days. Rats were anaesthetised with pentobarbitone (30 mg (kg body weight) $^{-1}$ ) and the abdomen opened by a midline incision. The distal colon was flushed with cold Ringer solution. Thereafter it was ligated at both ends, and injected luminally with 20  $\mu\text{g}$  of cholera toxin (Sigma, USA) in 1 ml of Ringer solution. The abdomen was then closed in layers, and the animal kept warm under a lamp. After 150 min of incubation, the abdomen was reopened and the colon removed after exsanguination of the animal by section of the inferior vena cava. Two pieces of colon were cut from distal colon, after stripping the serosa and external muscular layers, taking care to avoid any lymph node in the area.

### Rabbit distal colon

Male New Zealand White rabbits weighing 2.5–3 kg were maintained on standard diet *ad libitum* with free access to water. Under pentobarbitone sodium (30 mg (kg body wt) $^{-1}$ ) and ketamine (25 mg (kg body wt) $^{-1}$ ) anaesthesia the rabbit abdomen was opened through a 5 cm long midline incision just below the xiphisternum. All experiments were done at 16:00 h, since  $\text{Na}^+$  transport in this tissue shows circadian variation due to the influence of aldosterone (Hoffmann & Clauss, 1989). For cholera toxin pretreatment, the distal 15 cm of colon was flushed with cold Ringer solution, and a loop was constructed in which 6 ml of Ringer solution, containing 100  $\mu\text{g}$  of cholera toxin (or Ringer solution without cholera toxin, as control) was instilled. The abdomen was then closed in layers, and animals maintained with

**Table 1. Unidirectional and net fluxes of Na<sup>+</sup> and Cl<sup>-</sup> across rat distal colon: effect of mucosal amiloride and butyrate**

	<i>G</i>	<i>I</i> <sub>SC</sub>	<i>J</i> <sub>Na,ms</sub>	<i>J</i> <sub>Na,sm</sub>	<i>J</i> <sub>Na,net</sub>	<i>J</i> <sub>Cl,ms</sub>	<i>J</i> <sub>Cl,sm</sub>	<i>J</i> <sub>Cl,net</sub>
Control								
Basal	7.7 ± 0.4	1.4 ± 0.2	6.8 ± 0.4	3.7 ± 0.2	3.0 ± 0.2	4.6 ± 0.5	1.7 ± 0.4	2.9 ± 0.4
Amiloride	7.9 ± 0.4 <sup>n.s.</sup>	1.3 ± 0.2 <sup>n.s.</sup>	6.2 ± 0.3 <sup>n.s.</sup>	3.8 ± 0.3 <sup>n.s.</sup>	2.4 ± 0.3 <sup>n.s.</sup>	5.1 ± 0.5 <sup>n.s.</sup>	2.3 ± 0.5 <sup>n.s.</sup>	2.8 ± 0.3 <sup>n.s.</sup>
Butyrate								
Basal	9.3 ± 0.4 <sup>†</sup>	0.7 ± 0.1 <sup>‡</sup>	11.0 ± 0.6 <sup>§</sup>	5.0 ± 0.4 <sup>*</sup>	6.1 ± 0.5 <sup>§</sup>	8.8 ± 0.7 <sup>§</sup>	3.8 ± 0.2 <sup>§</sup>	5.0 ± 0.6 <sup>*</sup>
Amiloride	9.1 ± 0.4 <sup>n.s.</sup>	0.6 ± 0.1 <sup>n.s.</sup>	11.3 ± 0.8 <sup>n.s.</sup>	4.7 ± 0.6 <sup>n.s.</sup>	6.7 ± 0.7 <sup>n.s.</sup>	9.0 ± 1.0 <sup>n.s.</sup>	3.8 ± 0.5 <sup>n.s.</sup>	5.2 ± 0.6 <sup>n.s.</sup>

*G*, conductance (mS cm<sup>-2</sup>); *I*<sub>SC</sub>, short-circuit current (μmol h<sup>-1</sup> cm<sup>-2</sup>); *J*<sub>Na,ms</sub> and *J*<sub>Cl,ms</sub> are Na<sup>+</sup> and Cl<sup>-</sup> flux in the mucosa-to-serosa direction; *J*<sub>Na,sm</sub> and *J*<sub>Cl,sm</sub> are Na<sup>+</sup> and Cl<sup>-</sup> flux in the serosa-to-mucosa direction; *J*<sub>Na,net</sub> and *J*<sub>Cl,net</sub> are net flux of Na<sup>+</sup> and Cl<sup>-</sup> (all fluxes in μmol h<sup>-1</sup> cm<sup>-2</sup>). Control: *J*<sub>net</sub> (*n* = 8) and *G* (*n* = 16); butyrate: *J*<sub>net</sub> (*n* = 11) and *G* (*n* = 22). Amiloride was always added to the mucosal bath solution after three 15 min flux periods. Significance refers to comparison of post-amiloride with the basal condition in each group, and also between the two basal conditions; \**P* < 0.05; †*P* < 0.01; ‡*P* < 0.001; §*P* < 0.0001; n.s., not significantly different. Values are given as means ± S.E.M.

anaesthesia under a lamp. The abdomen was reopened after 30 min and the loop removed after the animals were killed by exsanguination. The colon was then cut longitudinally, flushed with cold, oxygenated Ringer solution, and fixed on a wax block. The serosa and outer muscular layers were stripped by blunt dissection under a dissecting microscope. The animal protocols were approved by the Institutional Animal Ethics Committee of the Christian Medical College, Vellore.

#### Ion flux measurements

The isolated mucosal sheet was mounted in Lucite flux chambers (World Precision Instruments, Sarasota, FL, USA) exposing 1.13 cm<sup>2</sup> surface area. Both sides of the tissue were bathed in the same solution. All experiments were performed at 37 °C. Solution mixing, oxygenation and pH (7.2–7.4) were maintained by continuous bubbling with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Potential difference (PD) and short-circuit current (*I*<sub>SC</sub>) were measured using an automatic voltage/current clamp apparatus (DVC-1000, World Precision Instruments). Conductance (*G*) expressed as mS cm<sup>-2</sup> was derived through application of Ohm's law. Unidirectional mucosa-to-serosa and serosa-to-mucosa fluxes of Na<sup>+</sup> (*J*<sub>Na,ms</sub> and *J*<sub>Na,sm</sub>) and Cl<sup>-</sup> (*J*<sub>Cl,ms</sub> and *J*<sub>Cl,sm</sub>) were measured by adding radioactive isotopes, <sup>22</sup>Na and <sup>36</sup>Cl, to either the mucosal or serosal side as appropriate. Tissues were paired for ion flux studies on the basis of differences in conductance no greater than 10%. After equilibration, the zero time PD and *I*<sub>SC</sub> were noted. One millilitre of solution was withdrawn from the (cold) side opposite to the hot side, and replaced with 1 ml of unlabelled solution. From then on, the tissue was continuously clamped at zero voltage, to eliminate passive flow of ions due to electrochemical drag. After two time periods of 15 min each, bumetanide (100 μg in 0.1% DMSO final concentration, to serosal bath solution) or amiloride (10 μM in 0.1% DMSO final concentration, to mucosal bath solution) were added in some experiments as noted. After such perturbations, 15 min was allowed for equilibration before measuring steady-state fluxes. Samples were taken from the cold side after each flux period of 15 min, and fluxes calculated using the mean of two flux periods. At the end of the experiment, 100 μl of solution was withdrawn from the hot side to estimate total radioactivity added to the side. <sup>22</sup>Na activity was measured using a gamma counter (CompuGamma, LKB, Sweden), while <sup>36</sup>Cl was measured using a liquid scintillation counter (RackBeta, LKB, Sweden) after appropriate correction for <sup>22</sup>Na. Unidirectional fluxes were

calculated using standard formulae, and expressed as μmol h<sup>-1</sup> cm<sup>-2</sup>. Net flux (*J*<sub>net</sub>) was calculated as the difference between *J*<sub>ms</sub> and *J*<sub>sm</sub> fluxes across tissue pairs. All experiments were performed under short-circuit conditions. Both sides of the rat tissue were bathed in bicarbonate (HCO<sub>3</sub><sup>-</sup>)-free Ringer solution in the first set of experiments, and then HCO<sub>3</sub><sup>-</sup>-free Ringer solution containing 25 mM butyrate in the second set of experiments, while rabbit tissues were bathed in Ringer solution. HCO<sub>3</sub><sup>-</sup>-free Ringer solution contained (mmol l<sup>-1</sup>): Na<sup>+</sup> 140, Cl<sup>-</sup> 119.8, K<sup>+</sup> 5.2, HPO<sub>4</sub><sup>-</sup> 2.4, H<sub>2</sub>PO<sub>4</sub><sup>-</sup> 0.4, Mg<sup>2+</sup> 1.2, Ca<sup>2+</sup> 1.2, isethionate 25 and glucose 10. Butyrate Ringer solution contained (mmol l<sup>-1</sup>): Na<sup>+</sup> 140, Cl<sup>-</sup> 119.8, K<sup>+</sup> 5.2, HPO<sub>4</sub><sup>-</sup> 2.4, H<sub>2</sub>PO<sub>4</sub><sup>-</sup> 0.4, Mg<sup>2+</sup> 1.2, Ca<sup>2+</sup> 1.2, butyrate 25 and glucose 10. HCO<sub>3</sub><sup>-</sup>-free Ringer solution was used in the rat in order to avoid HCO<sub>3</sub><sup>-</sup>-stimulated Na<sup>+</sup> and Cl<sup>-</sup> absorption.

#### Statistical analysis

Results are given as means ± S.E.M. Student's two-tailed *t* test (unpaired or paired as appropriate) was used to determine significance of differences. *P* < 0.05 was considered significant.

## RESULTS

#### Studies in rat distal colon

**Basal Na<sup>+</sup> and Cl<sup>-</sup> flux.** In HCO<sub>3</sub><sup>-</sup>-free Ringer solution, net Na<sup>+</sup> (3.04 ± 0.2 μmol h<sup>-1</sup> cm<sup>-2</sup>) and Cl<sup>-</sup> (2.9 ± 0.4 μmol h<sup>-1</sup> cm<sup>-2</sup>) absorption were of similar magnitude under basal conditions (Table 1). Addition of 10 μM amiloride did not result in any significant decrease in unidirectional flux or *J*<sub>Na,net</sub> and *J*<sub>Cl,net</sub>, or in *I*<sub>SC</sub>, indicating that amiloride-sensitive sodium channels did not contribute to this absorption.

**Effect of butyrate on basal Na<sup>+</sup> and Cl<sup>-</sup> fluxes.** Net Na<sup>+</sup> and Cl<sup>-</sup> absorption were significantly higher (*P* < 0.0001 and *P* < 0.01, respectively) in studies with butyrate-containing solution, compared with the absence of butyrate (Table 1). Increase in net absorption was due largely to significant increase in mucosa-to-serosa flux of both Na<sup>+</sup> (*P* < 0.0001) and Cl<sup>-</sup> (*P* < 0.0001). *I*<sub>SC</sub> was significantly lower when compared with HCO<sub>3</sub><sup>-</sup>-free Ringer solution (*P* < 0.001). Addition of 10 μM amiloride did not bring about any significant change in unidirectional or net fluxes of Na<sup>+</sup> or Cl<sup>-</sup>, indicating that butyrate stimulation of Na<sup>+</sup> and Cl<sup>-</sup>

**Table 2. Unidirectional and net fluxes of Na<sup>+</sup> and Cl<sup>-</sup> across distal colon of hyperaldosteronaemic rats: effect of amiloride and butyrate**

	<i>G</i>	<i>I</i> <sub>SC</sub>	<i>J</i> <sub>Na,ms</sub>	<i>J</i> <sub>Na,sm</sub>	<i>J</i> <sub>Na,net</sub>	<i>J</i> <sub>Cl,ms</sub>	<i>J</i> <sub>Cl,sm</sub>	<i>J</i> <sub>Cl,net</sub>
Control								
Basal	8.3 ± 0.4	2.4 ± 0.2	7.6 ± 0.8	4.2 ± 0.7	3.4 ± 0.3	4.8 ± 0.6	5.0 ± 0.5	-0.2 ± 0.2
Amiloride	8.7 ± 0.4§	0.4 ± 0.1§	5.2 ± 0.9*	5.0 ± 1.0 <sup>n.s.</sup>	0.2 ± 0.2§	5.9 ± 0.3 <sup>n.s.</sup>	5.8 ± 0.3 <sup>n.s.</sup>	0.1 ± 0.1 <sup>n.s.</sup>
Butyrate								
Basal	9.6 ± 0.5 <sup>n.s.</sup>	1.7 ± 0.3 <sup>n.s.</sup>	9.6 ± 0.5*	3.7 ± 0.4 <sup>n.s.</sup>	5.9 ± 0.2§	6.1 ± 0.4 <sup>n.s.</sup>	4.0 ± 0.3 <sup>n.s.</sup>	2.1 ± 0.3§
Amiloride	11.1 ± 0.5§	0.1 ± 0.2†	8.0 ± 0.8 <sup>n.s.</sup>	5.4 ± 0.7 <sup>n.s.</sup>	2.7 ± 0.4§	7.0 ± 0.8 <sup>n.s.</sup>	4.1 ± 0.5 <sup>n.s.</sup>	2.9 ± 0.5 <sup>n.s.</sup>

For both control and butyrate studies, the values shown are means ± S.E.M. of eight tissue pairs. Amiloride was always added to the mucosal bath solution after three 15 min flux periods. See Table 1 for further details and definitions.

**Table 3. Unidirectional and net fluxes of Na<sup>+</sup> and Cl<sup>-</sup> across distal colon of rats pre-exposed to cholera toxin (CT): effect of butyrate and bumetanide**

	<i>G</i>	<i>I</i> <sub>SC</sub>	<i>J</i> <sub>Na,ms</sub>	<i>J</i> <sub>Na,sm</sub>	<i>J</i> <sub>Na,net</sub>	<i>J</i> <sub>Cl,ms</sub>	<i>J</i> <sub>Cl,sm</sub>	<i>J</i> <sub>Cl,net</sub>
Control								
Post-CT	10.2 ± 0.5	3.0 ± 0.2	6.2 ± 0.5	7.5 ± 0.4	-1.3 ± 0.3	13.7 ± 1.0	16.7 ± 1.0	-3.0 ± 0.4
Bumetanide	11.1 ± 0.5§	1.8 ± 0.1‡	7.4 ± 0.2*	5.1 ± 0.3‡	2.3 ± 0.3§	14.5 ± 0.5 <sup>n.s.</sup>	10.2 ± 0.5§	4.3 ± 0.3§
Butyrate								
Post-CT	8.3 ± 0.3†	1.0 ± 0.6§	10.4 ± 0.7§	4.1 ± 0.5§	6.3 ± 0.6§	19.8 ± 0.8§	13.2 ± 0.6†	6.6 ± 0.9§
Bumetanide	8.3 ± 0.2 <sup>n.s.</sup>	0.5 ± 0.1§	9.9 ± 0.6 <sup>n.s.</sup>	3.8 ± 0.6 <sup>n.s.</sup>	6.1 ± 0.8 <sup>n.s.</sup>	20.2 ± 0.9 <sup>n.s.</sup>	14.1 ± 0.6 <sup>n.s.</sup>	6.1 ± 0.6 <sup>n.s.</sup>

For both control and butyrate studies, values shown are means ± S.E.M. of nine matched tissue pairs. Bumetanide was always added to the serosal reservoir after three 15 min flux periods. See Table 1 for further details and definitions.

absorption was not inhibited by this concentration of amiloride.

**Basal Na<sup>+</sup> and Cl<sup>-</sup> fluxes in hyperaldosteronaemic rat distal colon.** An increase in *I*<sub>SC</sub> was noted in hyperaldosteronaemic rat colon when compared with normal rat colon ( $P < 0.0001$ ) (Table 2). Unidirectional flux and net absorption of Na<sup>+</sup> did not show any significant difference from the control rats. Unidirectional Cl<sup>-</sup> fluxes were approximately equal, leading to nearly absent net Cl<sup>-</sup> movement across the mucosa. Thus *J*<sub>Cl,sm</sub> was significantly increased ( $P < 0.0001$ ) and *J*<sub>Cl,net</sub> significantly decreased ( $P < 0.0001$ ) compared with normal rat colon. Addition of amiloride resulted in a significant reduction of *I*<sub>SC</sub> ( $P < 0.0001$ ), and in *J*<sub>Na,net</sub> ( $P < 0.0001$ ), the latter largely due to reduction in *J*<sub>Na,ms</sub> ( $P < 0.017$ ), indicating that amiloride-sensitive Na<sup>+</sup> channels were present in hyperaldosteronaemic rats. Net Cl<sup>-</sup> absorption on the other hand did not show any significant change.

**Effect of butyrate on Na<sup>+</sup> and Cl<sup>-</sup> fluxes in hyperaldosteronaemic rat distal colon.** In butyrate solution there was a significant increase in *J*<sub>Na,net</sub> ( $P < 0.0001$ ) and *J*<sub>Cl,net</sub> ( $P < 0.0001$ ) compared with control studies using butyrate-free solution (Table 2). This was associated with a decrease in *I*<sub>SC</sub> ( $P < 0.016$ ). Addition of amiloride resulted in near abolition of the *I*<sub>SC</sub> ( $P < 0.002$ ), and a significant reduction in *J*<sub>Na,net</sub> ( $P < 0.0001$ ), but no significant change in *J*<sub>Cl,net</sub> ( $P$ , n.s.). The reduction in *I*<sub>SC</sub> and *J*<sub>Na,net</sub> was comparable in magnitude to that seen in the absence of butyrate. However, unlike the control, there was a

significant residual *J*<sub>Na,net</sub> in the presence of butyrate, which equalled *J*<sub>Cl,net</sub>, indicating electroneutral NaCl absorption.

**Effect of butyrate in cholera toxin-treated rat distal colon.** Cholera toxin-exposed colon in butyrate-free solution demonstrated reversal of net Na<sup>+</sup> and Cl<sup>-</sup> absorption to net Na<sup>+</sup> and Cl<sup>-</sup> secretion (Table 3). This is consistent with inhibition of electroneutral NaCl absorption with induction of active Cl<sup>-</sup> secretion. Addition of serosal bumetanide resulted in a decreased *I*<sub>SC</sub> ( $P < 0.0001$ ) along with markedly reduced *J*<sub>Cl,sm</sub> ( $P < 0.001$ ) and *J*<sub>Na,sm</sub>, due to inhibition of basolateral Na<sup>+</sup>-K<sup>+</sup>-2Cl<sup>-</sup> cotransport (Table 3). The increase in *J*<sub>Cl,net</sub> ( $P < 0.0001$ ) seen after addition of bumetanide was twice that for *J*<sub>Na,net</sub> ( $P < 0.0001$ ), in keeping with the stoichiometry of Na<sup>+</sup>-K<sup>+</sup>-2Cl<sup>-</sup> cotransport. Cholera toxin-exposed tissue, in butyrate-containing solution, was associated with a significantly lower *I*<sub>SC</sub> ( $P < 0.0001$ ) and an increase in net Na<sup>+</sup> ( $P < 0.0001$ ) and Cl<sup>-</sup> ( $P < 0.0001$ ) absorption compared with butyrate-free solution. Addition of bumetanide did not further change Na<sup>+</sup> ( $P$ , n.s.) or Cl<sup>-</sup> ( $P$ , n.s.) absorption. Therefore, butyrate not only increased net Na<sup>+</sup> and Cl<sup>-</sup> absorption compared with control, but also completely inhibited cholera toxin-induced secretion.

**Effect of butyrate in cholera toxin-treated hyperaldosteronaemic rat distal colon.** In Ringer solution, net Na<sup>+</sup> absorption was noted in cholera toxin-treated tissue exposed to butyrate-free solution ( $P < 0.01$ ) (Table 4), and was similar in magnitude to net Na<sup>+</sup> absorption from hyperaldosteronaemic rat colon not treated with cholera toxin ( $P$ , n.s.). Cholera toxin induced net Cl<sup>-</sup> secretion,



**Table 4. Unidirectional and net fluxes of Na<sup>+</sup> and Cl<sup>-</sup> across distal colon of hyperaldosteronaemic rats pre-exposed to cholera toxin: effect of butyrate and bumetanide**

	<i>G</i>	<i>I</i> <sub>SC</sub>	<i>J</i> <sub>Na,ms</sub>	<i>J</i> <sub>Na,sm</sub>	<i>J</i> <sub>Na,net</sub>	<i>J</i> <sub>Cl,ms</sub>	<i>J</i> <sub>Cl,sm</sub>	<i>J</i> <sub>Cl,net</sub>
Control								
Post-CT	9.7 ± 0.6	3.2 ± 0.2	7.4 ± 0.7	4.3 ± 0.6	3.2 ± 0.4	12.2 ± 0.7	13.4 ± 0.7	-1.2 ± 0.5
Bumetanide	11.5 ± 0.6§	1.1 ± 0.1§	8.2 ± 0.9 <sup>n.s.</sup>	5.3 ± 0.8*	3.0 ± 0.9 <sup>n.s.</sup>	13.8 ± 0.9*	10.1 ± 0.8*	3.7 ± 1.0‡
Butyrate								
Post-CT	9.0 ± 0.5 <sup>n.s.</sup>	1.3 ± 0.1§	10.2 ± 1.2 <sup>n.s.</sup>	4.1 ± 0.5 <sup>n.s.</sup>	6.2 ± 0.9*	19.3 ± 1.4‡	12.7 ± 0.7 <sup>n.s.</sup>	6.6 ± 1.0‡
Bumetanide	10.3 ± 0.5†	0.8 ± 0.1§	11.1 ± 0.8 <sup>n.s.</sup>	5.6 ± 0.8†	5.6 ± 0.8 <sup>n.s.</sup>	20.2 ± 1.1 <sup>n.s.</sup>	12.9 ± 0.8 <sup>n.s.</sup>	7.3 ± 0.6 <sup>n.s.</sup>

Values shown are means ± S.E.M. of matched tissue pairs; control, *n* = 8 pairs and butyrate, *n* = 9 pairs. Bumetanide was always added to the serosal reservoir after three flux periods. See Table 1 for further details and definitions.

**Table 5. Effect of butyrate and amiloride on unidirectional and net fluxes of Na<sup>+</sup> and Cl<sup>-</sup> across rabbit distal colon**

	<i>G</i>	<i>I</i> <sub>SC</sub>	<i>J</i> <sub>Na,ms</sub>	<i>J</i> <sub>Na,sm</sub>	<i>J</i> <sub>Na,net</sub>	<i>J</i> <sub>Cl,ms</sub>	<i>J</i> <sub>Cl,sm</sub>	<i>J</i> <sub>Cl,net</sub>
Control	8.8 ± 0.4	2.4 ± 0.1	4.2 ± 0.3	2.4 ± 0.4	1.8 ± 0.2	5.7 ± 0.4	6.4 ± 0.4	-0.7 ± 0.4
Butyrate	6.9 ± 0.3§	0.9 ± 0.0§	4.3 ± 0.4 <sup>n.s.</sup>	1.4 ± 0.3*	2.9 ± 0.3*	4.4 ± 0.5 <sup>n.s.</sup>	4.4 ± 0.5†	0.0 ± 0.5 <sup>n.s.</sup>

For both control (*n* = 9) and butyrate (*n* = 12) studies, values shown are means ± S.E.M. of matched tissue pairs. See Table 1 for further details and definitions.

similar in magnitude to that seen in normal rat colon. These results are consistent with the observation that Na<sup>+</sup> absorption mediated through Na<sup>+</sup> channels (as in hyperaldosteronism) is not inhibited by cholera toxin, whereas Cl<sup>-</sup> secretion is induced by cholera toxin. On addition of bumetanide, there was no significant increase in Na<sup>+</sup> absorption, whereas net Cl<sup>-</sup> secretion was converted to net Cl<sup>-</sup> absorption (*P* < 0.001). The change in *I*<sub>SC</sub> following bumetanide was less than the change in *J*<sub>Cl,net</sub>. Hyperaldosteronism induces electrogenic K<sup>+</sup> secretion (Foster *et al.* 1984), and this will cause changes in *I*<sub>SC</sub> in a direction opposite to Cl<sup>-</sup> secretion. It is likely that bumetanide reduced K<sup>+</sup> and Cl<sup>-</sup> secretion simultaneously, accounting for the disparity in the *I*<sub>SC</sub> and *J*<sub>Cl,net</sub> response.

Studies in 25 mM butyrate showed increased net absorption of both Na<sup>+</sup> and Cl<sup>-</sup> due to increased *J*<sub>Na,ms</sub> and *J*<sub>Cl,ms</sub>, while *J*<sub>sm</sub> was comparable to control studies in the absence of cholera toxin. Butyrate thus stimulated both *J*<sub>Na,net</sub> (*P* < 0.01) and *J*<sub>Cl,net</sub> (*P* < 0.001), while preventing the increase in serosa-to-mucosa flux brought about by cholera toxin. *I*<sub>SC</sub> in butyrate solution was significantly lower (*P* < 0.0001) than in control. There was no further increase in net Na<sup>+</sup> or Cl<sup>-</sup> flux on addition of bumetanide.

### Studies in rabbit distal colon

**Basal Na<sup>+</sup> and Cl<sup>-</sup> fluxes in rabbit distal colon.** *J*<sub>Na,net</sub> of 1.8 ± 0.2 μmol h<sup>-1</sup> cm<sup>-2</sup> was observed in the basal state from Ringer solution. This was not accompanied by net Cl<sup>-</sup> absorption, and in fact a minimal net Cl<sup>-</sup> secretion (-0.7 ± 0.4 μmol h<sup>-1</sup> cm<sup>-2</sup>) was observed (Table 5). The observed *I*<sub>SC</sub> (2.4 ± 0.1 μmol h<sup>-1</sup> cm<sup>-2</sup>) could be accounted for by a combination of electrogenic Na<sup>+</sup> absorption and minimal electrogenic Cl<sup>-</sup> secretion. The virtual absence of electroneutral Na<sup>+</sup> absorption under basal conditions was

shown by the fact that 10<sup>-4</sup> M amiloride (a concentration that inhibits Na<sup>+</sup> channels) inhibited *J*<sub>Na,net</sub> by 78% (0.4 ± 0.2 μmol h<sup>-1</sup> cm<sup>-2</sup> post-amiloride, compared with 1.8 ± 0.1 μmol h<sup>-1</sup> cm<sup>-2</sup> in the basal state). This was accompanied by a change in *I*<sub>SC</sub> from 2.0 ± 0.1 μmol h<sup>-1</sup> cm<sup>-2</sup> in the basal state to 1.0 ± 0.1 μmol h<sup>-1</sup> cm<sup>-2</sup> after amiloride (*P* < 0.001).

**Effect of butyrate on basal Na<sup>+</sup> and Cl<sup>-</sup> fluxes.** As shown in Table 5, in the presence of 25 mM butyrate a significant increase was noted in *J*<sub>Na,net</sub> (2.9 ± 0.3 μmol h<sup>-1</sup> cm<sup>-2</sup>) compared with the basal values (*P* < 0.02). This was secondary to a significant decrease (*P* < 0.04) in *J*<sub>Na,sm</sub> while *J*<sub>Na,ms</sub> was almost unaltered. *J*<sub>Cl,sm</sub> decreased in the presence of butyrate, but *J*<sub>Cl,ms</sub> and *J*<sub>Cl,net</sub> were not significantly affected by butyrate. A decrease in conductance was noted after addition of butyrate (*P* < 0.0001), and this could partially account for reduced *J*<sub>Na,sm</sub>. The fact that this was not accompanied by a comparable decrease in *J*<sub>Na,ms</sub> indicates that there was active Na<sup>+</sup> absorption. Although butyrate increased *J*<sub>Na,net</sub>, it decreased the *I*<sub>SC</sub> (0.9 ± 0.0 μmol h<sup>-1</sup> cm<sup>-2</sup>), indicating that the stimulation of net Na<sup>+</sup> absorption was through an electroneutral mechanism. This was confirmed by the observation that 10<sup>-4</sup> M amiloride inhibited *J*<sub>Na,net</sub> only partially (2.0 ± 0.4 μmol h<sup>-1</sup> cm<sup>-2</sup> post-amiloride, compared with 3.1 ± 0.2 μmol h<sup>-1</sup> cm<sup>-2</sup> in the basal state) in the presence of butyrate. This was accompanied by a change in *I*<sub>SC</sub> from 0.9 ± 0.1 μmol h<sup>-1</sup> cm<sup>-2</sup> in the basal state to -0.5 ± 0.1 μmol h<sup>-1</sup> cm<sup>-2</sup> after amiloride (*P* < 0.0001). Fluxes in Cl<sup>-</sup>-free Ringer solution indicated that butyrate stimulation of Na<sup>+</sup> absorption was not dependent on presence of Cl<sup>-</sup> in the medium (Fig. 1).

**Na<sup>+</sup> and Cl<sup>-</sup> fluxes in cholera toxin-treated rabbit distal colon.** Unidirectional and *J*<sub>Na,net</sub> after exposure to cholera

**Table 6. Unidirectional and net fluxes of Na<sup>+</sup> and Cl<sup>-</sup> across rabbit distal colon pre-incubated with cholera toxin (CT)**

	<i>G</i>	<i>I</i> <sub>SC</sub>	<i>J</i> <sub>Na,ms</sub>	<i>J</i> <sub>Na,sm</sub>	<i>J</i> <sub>Na,net</sub>	<i>J</i> <sub>Cl,ms</sub>	<i>J</i> <sub>Cl,sm</sub>	<i>J</i> <sub>Cl,net</sub>
Control								
Post-CT	8.2 ± 0.2	3.2 ± 0.3	4.3 ± 0.4	2.6 ± 0.3	1.7 ± 0.3	5.3 ± 0.7	9.8 ± 0.6	-4.5 ± 0.5
Post-bumetanide	8.6 ± 0.3 <sup>n.s.</sup>	0.8 ± 0.1§	5.3 ± 0.4 <sup>n.s.</sup>	2.8 ± 0.5 <sup>n.s.</sup>	2.5 ± 0.5 <sup>n.s.</sup>	8.6 ± 0.7‡	7.8 ± 0.9 <sup>n.s.</sup>	0.8 ± 0.4‡
Butyrate								
Post-CT	5.6 ± 0.2§	2.0 ± 0.1§	3.4 ± 0.5 <sup>n.s.</sup>	0.8 ± 0.2§	2.6 ± 0.4†	5.3 ± 0.6 <sup>n.s.</sup>	5.0 ± 0.6§	0.3 ± 0.5§
Post-bumetanide	5.0 ± 0.2§	0.6 ± 0.1§	4.3 ± 0.4 <sup>n.s.</sup>	1.4 ± 0.2 <sup>n.s.</sup>	2.9 ± 0.6 <sup>n.s.</sup>	5.6 ± 1.0 <sup>n.s.</sup>	4.1 ± 0.9 <sup>n.s.</sup>	1.5 ± 0.4 <sup>n.s.</sup>

For both control (*n* = 7) and butyrate (*n* = 8), values shown are means ± s.e.m. of matched tissue pairs. Bumetanide was always added to the serosal bath solution after three flux periods. See Table 1 for further details and definitions.

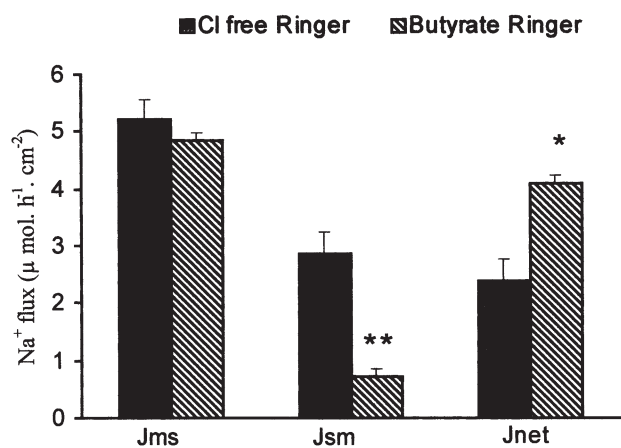
toxin were similar to fluxes in rabbit colon not exposed to cholera toxin (*P*, n.s.). This indicates a lack of suppression of electrogenic Na<sup>+</sup> absorption by cholera toxin. On the other hand, cholera toxin produced a significant increase in net Cl<sup>-</sup> secretion (Table 6) (*P* < 0.0001), which resulted from a significant increase (*P* = 0.0001) in *J*<sub>Cl,sm</sub> without any significant alteration in *J*<sub>Cl,ms</sub>. This was accompanied by an increase in *I*<sub>SC</sub> to 3.2 ± 0.3 μmol h<sup>-1</sup> cm<sup>-2</sup> (*P* = 0.0001). The magnitude of the increase in *I*<sub>SC</sub> did not completely account for the sum of *J*<sub>Na,net</sub> and *J*<sub>Cl,net</sub>. Prostaglandin E<sub>2</sub>, possibly acting through cAMP, is known to induce simultaneous, and electrogenic, K<sup>+</sup> and Cl<sup>-</sup> secretion in rabbit descending colon (Halm & Frizzell, 1986; Roden *et al.* 1992), and both these are inhibited by bumetanide. It is therefore likely that the *I*<sub>SC</sub> response to Cl<sup>-</sup> secretion was blunted by the presence of concomitant K<sup>+</sup> secretion induced by cAMP. Bumetanide addition to the serosal side did not significantly affect unidirectional or net *J*<sub>Na</sub> (*P*, n.s.).

On the other hand, bumetanide totally inhibited net Cl<sup>-</sup> secretion (*P* < 0.001) and resulted in minimal net Cl<sup>-</sup> absorption. This was due to increased *J*<sub>Cl,ms</sub> (*P* = 0.001) while *J*<sub>Cl,sm</sub> was not significantly affected. Bumetanide inhibited *I*<sub>SC</sub> significantly, and reduced residual flux from 3.0 to 1.2 μmol h<sup>-1</sup> cm<sup>-2</sup>, probably due to simultaneous inhibition of Cl<sup>-</sup> and K<sup>+</sup> secretion.

**Effect of butyrate on Na<sup>+</sup> and Cl<sup>-</sup> fluxes in cholera toxin-treated rabbit distal colon.** In cholera toxin-treated rabbit distal colon, the presence of butyrate increased net Na<sup>+</sup> absorption significantly (*P* = 0.004) compared with control colon (Table 6). This was due to decreased *J*<sub>Na,sm</sub> (*P* < 0.0001) while *J*<sub>Na,ms</sub> was not significantly altered. There was a significant decrease in conductance (*P* < 0.0001) when compared with that in the Ringer solution. In the presence of butyrate, net Cl<sup>-</sup> absorption was noted in cholera toxin-treated colon (*P* < 0.0001, compared with control). This was due to significant reduction in *J*<sub>Cl,sm</sub> (*P* = 0.0001), while *J*<sub>Cl,ms</sub> remained unaltered compared with that in the absence of butyrate. Addition of bumetanide did not significantly alter unidirectional or net fluxes of either Na<sup>+</sup> or Cl<sup>-</sup> in these experiments. These results are consistent with an inhibitory effect of butyrate on active Cl<sup>-</sup> secretion induced by cholera toxin.

## DISCUSSION

Active absorption of sodium by the colon is necessary for water conservation by the body in health, and becomes critical in disease conditions characterised by excessive fluid losses from the body. The absorptive process involves either Na<sup>+</sup> channels (electrogenic absorption) or Na<sup>+</sup>-H<sup>+</sup> exchange (electroneutral absorption). Marked regional and species variations exist in the distribution of these transport processes. While Na<sup>+</sup>-H<sup>+</sup> exchange predominates in rat distal colon, and Na<sup>+</sup> channel activity predominates in rabbit distal colon, there is a mixture of the two processes in human distal colon (Binder *et al.* 1987; Sellin & De Soignie, 1987; Sandle, 1989). Short chain fatty acids (SCFA) stimulate active Na<sup>+</sup> absorption from the colon of many mammalian species including man, and this has



**Figure 1. Effect of Cl<sup>-</sup> withdrawal from the bath solution on unidirectional and net Na<sup>+</sup> fluxes in rabbit descending colon**

Both the solutions used for the above study contained equimolar amounts of isethionate in place of Cl<sup>-</sup>. Basal as well as butyrate-stimulated Na<sup>+</sup> absorption took place even in the absence of Cl<sup>-</sup>. Butyrate induced a significant increase in *J*<sub>Na,net</sub> (\**P* = 0.006) and *J*<sub>Na,sm</sub> (\*\**P* = 0.002) compared with basal values.

been shown to be secondary to stimulation of electro-neutral NaCl absorption (Binder *et al.* 1989). Butyrate-dependent NaCl absorption is not inhibited by cAMP (Binder & Mehta, 1990; Krishnan *et al.* 1999). There is also evidence to suggest that butyrate inhibits active Cl<sup>-</sup> secretion, the basis of secretory diarrhoea, in rat colon and secretory T84 cells (Dagher *et al.* 1996; Matthews *et al.* 1998). The studies reported here suggest that augmentation of NaCl absorption and prevention of Cl<sup>-</sup> secretion are simultaneous and independent effects of butyrate.

As demonstrated earlier, butyrate-stimulated electro-neutral NaCl absorption in rat distal colon was not inhibited by 10<sup>-4</sup> M amiloride. In the same tissue, electrogenic Cl<sup>-</sup> secretion induced by cholera toxin was prevented completely by butyrate, and there was no significant change in fluxes after addition of bumetanide to butyrate-exposed tissues. This observation confirms the earlier finding of the antisecretory effect of butyrate in rat colon (Dagher *et al.* 1996). The increase in net Na<sup>+</sup> absorption after butyrate was also observed in cholera toxin-treated colon and after addition of bumetanide, showing that this effect was independent of the effect of butyrate on Cl<sup>-</sup> secretion. We also demonstrate here that butyrate increased electroneutral Na<sup>+</sup> absorption in rabbit distal colon, a tissue not previously examined in this regard, and which lacks basal Na<sup>+</sup>-H<sup>+</sup> exchange. Again, in rabbit colon, cholera toxin-induced Cl<sup>-</sup> secretion was completely prevented by addition of butyrate to the medium.

Several of our findings appear contradictory, but may be explained further. In the rat distal colon, butyrate augmented net Na<sup>+</sup> and Cl<sup>-</sup> absorption by increasing  $J_{ms}$  of both Na<sup>+</sup> and Cl<sup>-</sup>, indicating that it stimulated inward fluxes of these ions. On the other hand, in the rabbit distal colon, butyrate augmented net Na<sup>+</sup> absorption principally by reducing  $J_{Na,sm}$ . Under basal conditions,  $J_{Na,sm}$  in the colon is generally considered to be a reflection of paracellular permeability. Butyrate reduced tissue conductance by 22%, and this reduction in paracellular permeability would probably account for half the reduction in  $J_{Na,sm}$  following butyrate. Reduced paracellular permeability would have concurrently reduced the passive component of  $J_{Na,ms}$ , and this reduction probably masked an increase in the active component of  $J_{Na,ms}$  in the presence of butyrate. Butyrate has been shown to reduce paracellular permeability in intestinal epithelial cell monolayers (Mariadason *et al.* 1997). An inhibitory effect of butyrate on basal Na<sup>+</sup> secretion in the colon would also have to be considered as a possibility. Na<sup>+</sup> secretion has not been reported under basal conditions in the colon. However, Na<sup>+</sup> secretion, largely coupled to HCO<sub>3</sub><sup>-</sup> secretion, may be induced by cAMP in rabbit proximal colon in the presence of high extracellular Na<sup>+</sup> concentrations (Hyun *et al.* 1994).

In rabbit distal colon, the presence of butyrate was associated with a decrease in  $I_{SC}$  while net Na<sup>+</sup> flux increased significantly, indicating that inhibition of Na<sup>+</sup> channels (Abriel & Horisberger, 1999; Chalfant *et al.* 1999) may have occurred alongside stimulation of Na<sup>+</sup>-H<sup>+</sup> exchange. As rabbit distal colon expresses only NHE2 and not NHE3 (Hoogerwerf *et al.* 1996), it is possible that the butyrate-dependent Na<sup>+</sup>-H<sup>+</sup> exchange occurred via NHE2. In rabbit distal colon, cholera toxin did not affect net Na<sup>+</sup> absorption, demonstrating that this process was not sensitive to cAMP, unlike Na<sup>+</sup>-H<sup>+</sup> exchange (McSwine *et al.* 1998; Zizak *et al.* 1999). Cholera toxin did induce net Cl<sup>-</sup> secretion by increasing  $J_{Cl,sm}$ , which could be completely inhibited by bumetanide, which blocks Na<sup>+</sup>-K<sup>+</sup>-2Cl<sup>-</sup> cotransporter activity. Inhibition of Na<sup>+</sup>-K<sup>+</sup>-2Cl<sup>-</sup> cotransport by bumetanide also resulted in an increased  $J_{Na,net}$ , which possibly reflects compensation of reduced intracellular Na<sup>+</sup> concentrations by increased apical Na<sup>+</sup> entry. However, for reasons that are not clear, the enhanced Na<sup>+</sup> and Cl<sup>-</sup> absorption values did not exactly coincide with the known stoichiometry for Na<sup>+</sup>-K<sup>+</sup>-2Cl<sup>-</sup> cotransport. In rabbit distal colon the magnitude of the increase in  $I_{SC}$  after cholera toxin did not correspond to the sum of Na<sup>+</sup> absorption and Cl<sup>-</sup> secretion. Active electrogenic K<sup>+</sup> secretion in the colon is stimulated by PGE2 and cAMP (Halm & Frizzell, 1986; Roden *et al.* 1992; Merlin *et al.* 1995; Grotjohann *et al.* 1998). This could explain the high basal residual ion flux of 3.0 μmol h<sup>-1</sup> cm<sup>-2</sup> seen in rabbit colon after exposure to cholera toxin.

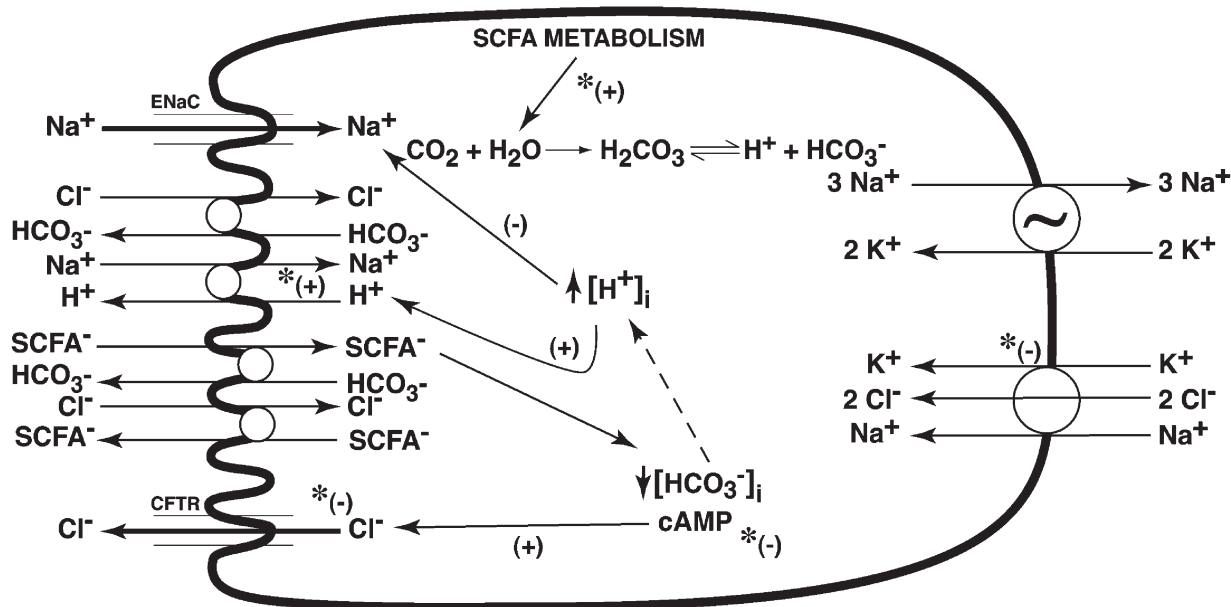
In rabbit colon, substitution of Cl<sup>-</sup> with isethionate (an unabsorbed anion) did not affect either basal or butyrate-dependent Na<sup>+</sup> absorption. This is not surprising since Na<sup>+</sup> absorption from rabbit descending colon is predominantly through Na<sup>+</sup> channels. The lack of dependence of butyrate-dependent Na<sup>+</sup> absorption on Cl<sup>-</sup> suggests that there is no necessity for linkage with Cl<sup>-</sup> absorption of butyrate-stimulated Na<sup>+</sup>-H<sup>+</sup> exchange. It is likely that butyrate entry into the cell (either via SCFA<sup>-</sup>-HCO<sub>3</sub><sup>-</sup> exchange or by diffusion of unionized butyrate) reduces intracellular pH and that this secondarily stimulates Na<sup>+</sup>-H<sup>+</sup> exchange (Gonda *et al.* 1999).

The pattern of Na<sup>+</sup> and Cl<sup>-</sup> absorption was also studied in hyperaldosteronaemic rat distal colon, a condition that resulted in significant increase in amiloride-inhibitable Na<sup>+</sup> current and flux. In Ringer solution, amiloride almost totally inhibited the  $I_{SC}$  and  $J_{Na,net}$ , indicating the presence of amiloride-inhibitable Na<sup>+</sup> channels in hyperaldosteronaemic rat distal colon. This colon resembled rabbit distal colon in demonstrating electrogenic Na<sup>+</sup> absorption and lacking Cl<sup>-</sup> absorption. In fact, minimal net Cl<sup>-</sup> secretion was observed. Butyrate increased Na<sup>+</sup> and Cl<sup>-</sup> absorption from salt-depleted rat distal colon, and this was associated with a significant decrease in  $I_{SC}$  ( $P < 0.02$ ).

Amiloride almost completely abolished the  $I_{SC}$  and produced a significant decrease in  $J_{Na,net}$ , but did not totally inhibit it, unlike the rabbit distal colon. This could be attributable to residual electroneutral  $Na^+$  absorption in the salt-depleted animals, or may reflect different sensitivity of the  $Na^+-H^+$  exchanger isoforms to amiloride. Studies have shown that rat distal colon has both NHE2 and NHE3 isoforms, while rabbit distal colon has only the NHE2 isoform (Hoogerwerf *et al.* 1996). NHE2 is inhibited at  $10^{-4}$  M amiloride concentration while NHE3 is not (Counillon *et al.* 1993). Downregulation of NHE3, and to a lesser extent NHE2, has been reported in the distal colon of salt-depleted rats (Ikuma *et al.* 1999). It is possible that butyrate stimulated NHE2 or NHE3 expression (Musch *et al.* 2001), since butyrate is known to have an effect on gene expression.

Cholera toxin treatment of the rat distal colon inhibited electroneutral sodium chloride absorption, and induced net  $Cl^-$  secretion. Minimal net secretion of  $Na^+$  was also observed, and this may have been related to the extracellular  $Na^+$  concentration (Hyun *et al.* 1994). This was

associated with a significant increase in  $I_{SC}$  ( $P < 0.0001$ ). Electrogenic  $Cl^-$  secretion requires the co-ordinated activity of several ion transporters including the apical  $Cl^-$  channel and basolateral  $Na^+-K^+-2Cl^-$  cotransport,  $Na^+-K^+-ATPase$  and  $K^+$  channels (Hecht & Koutsouris, 1999). Of these,  $Na^+-K^+-2Cl^-$  cotransport contributes directly to the chloride exit at the apical end (Matthews *et al.* 1998; Marunaka *et al.* 1999). The role of  $Na^+-K^+-2Cl^-$  cotransport was systematically evaluated by addition of bumetanide to the serosal side. This resulted in total inhibition of the electrogenic  $Cl^-$  secretion and also made it absorptive with a mean difference of  $7.3 \mu\text{mol h}^{-1} \text{cm}^{-2}$  ( $P < 0.0001$ ). Unidirectional fluxes of chloride showed a decrease in  $J_{Cl,sm}$  with a mean difference of  $6.0 \mu\text{mol h}^{-1} \text{cm}^{-2}$  ( $P < 0.0001$ ) with little or no change in  $J_{Cl,ms}$ . Addition of bumetanide also inhibited net  $Na^+$  secretion with a mean difference of  $3.6 \mu\text{mol h}^{-1} \text{cm}^{-2}$ . Unlike in rabbit distal colon, this inhibition of  $Na^+$  and  $Cl^-$  secretion agreed well with the stoichiometry of  $Na^+-K^+-2Cl^-$  cotransport. As expected, along with the inhibition of the electrogenic  $Cl^-$  secretion there was a significant decrease in  $I_{SC}$  ( $P < 0.001$ ).



**Figure 2. Conceptual diagram of active  $Na^+$  and  $Cl^-$  transport in mammalian colonic epithelium**

Possible transporters involved are shown, but not all are present in the same cell (e.g. rabbit distal colon lacks  $Na^+-H^+$  exchange and  $Cl^-HCO_3^-$  exchange, while rat distal colon lacks  $Na^+$  channel).  $Na^+-H^+$  exchange may be coupled to  $Cl^-HCO_3^-$  exchange, or independently coupled to  $SCFA^-HCO_3^-$  and  $Cl^-SCFA^-$  exchanges (Rajendran & Binder, 1994). Butyrate absorption into the cell may occur via non-ionic diffusion, and dissociation of butyrate within the cell may increase intracellular  $H^+$  ( $[H^+]_i$ ) with stimulation of  $Na^+-H^+$  exchange. Butyrate  $-HCO_3^-$  exchange (Mascolo *et al.* 1991) across the apical membrane will also increase  $[H^+]_i$  and stimulate  $Na^+-H^+$  exchange across the apical membrane. Increased  $[H^+]_i$  will also inhibit  $Na^+$  absorption via  $Na^+$  channels (Chalfant *et al.* 1999).  $Cl^-$  secretion involves  $Cl^-$  channels at the apical membrane, and  $Na^+-K^+-2Cl^-$  cotransporter activity and sodium pump activity in the basolateral membrane. Inhibition of  $Cl^-$  secretion by butyrate may occur at any of these levels. Butyrate also inhibits cAMP generation by colonic epithelium (Krishnan *et al.* 1999). \* Potential levels at which butyrate may potentiate (+) or inhibit (-) processes that eventually affect net  $Na^+$  transport.



Exposure of cholera toxin-treated rat distal colon to butyrate stimulated  $J_{\text{Na,net}}$  and  $J_{\text{Cl,net}}$  absorption with a mean difference of  $7.7 \mu\text{mol h}^{-1} \text{cm}^{-2}$  ( $P < 0.0001$ ) and  $9.5 \mu\text{mol h}^{-1} \text{cm}^{-2}$  ( $P < 0.0001$ ). From unidirectional fluxes a selective increase of  $J_{\text{Na,ms}}$  ( $P < 0.0001$ ) and  $J_{\text{Cl,ms}}$  ( $P < 0.0001$ ) were noted. This could be explained by stimulation of electroneutral  $\text{Na}^+ - \text{H}^+$  exchange and  $\text{SCFA}^-$ -linked  $\text{Cl}^- - \text{HCO}_3^-$  exchange at the apical membrane. A decrease in  $J_{\text{Na,sm}}$  and  $J_{\text{Cl,sm}}$  could be due to the effect of butyrate on tight junctions. Addition of bumetanide was without any further increase in  $J_{\text{Na,net}}$  and  $J_{\text{Cl,net}}$ . It indicates that the increase in  $J_{\text{Na,net}}$  and  $J_{\text{Cl,net}}$  occurred not because of a decrease in flux at the basolateral end, but because of a definite increase of an electroneutral absorptive process at the apical end. The current model (Fig. 2) for SCFA-dependent  $\text{Na}^+$  and  $\text{Cl}^-$  absorption could explain this increased absorption. It predicts an increase in  $\text{Na}^+ - \text{H}^+$  and  $\text{Cl}^- - \text{SCFA}^-$  exchange, secondary to intracellular acidosis by  $\text{SCFA}^- - \text{HCO}_3^-$  exchange at the brush-border membrane of the colonic epithelial cell.

Cholera toxin treatment of hyperaldosteronaemic rats showed again that electrogenic  $\text{Na}^+$  absorption was not inhibited by cAMP. There was no significant difference in  $J_{\text{Cl,net}}$  between cholera toxin-treated control and cholera toxin-treated hyperaldosteronaemic rats.  $\text{Cl}^-$  secretion was therefore not associated with aldosterone status of the animal. Thus  $\text{Na}^+$  and  $\text{Cl}^-$  absorption are not coupled and take place independently of each other. Inhibition of  $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$  cotransport resulted in an increase in  $J_{\text{Cl,net}}$  ( $P < 0.001$ ), with no significant difference in  $J_{\text{Na,net}}$ .  $I_{\text{SC}}$  showed an even greater decrease and could be attributed to inhibition of electrogenic  $\text{Cl}^-$  secretion and decreased  $\text{K}^+$  secretion at the apical end. Butyrate significantly increased  $J_{\text{Na,net}}$  ( $P < 0.01$ ) and  $J_{\text{Cl,net}}$  ( $P < 0.001$ ) with a significant decrease in  $I_{\text{SC}}$  ( $P < 0.0001$ ), indicating that  $\text{Na}^+$  and  $\text{Cl}^-$  absorption was electrically neutral. Addition of bumetanide to the butyrate-exposed tissues did not produce further change in  $\text{Na}^+$  and  $\text{Cl}^-$  absorption, excluding a role for  $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$  cotransport. Thus, as in control, butyrate stimulation of  $J_{\text{Na,net}}$  and  $J_{\text{Cl,net}}$  in hyperaldosteronaemic rat distal colon occurred because of an increase of electroneutral absorption at the apical membrane.

Figure 2 depicts a pictorial model of active transport processes for  $\text{Na}^+$  and  $\text{Cl}^-$  in the mammalian colon, which is based on that suggested by Binder and co-workers. A number of points are shown (in Fig. 2) at which butyrate can modulate these transport processes, and these can exist in a particular tissue to a greater or lesser extent depending on the basal transport processes characteristic of each epithelium. In addition to direct interactions with transport processes, butyrate may also have effects on expression of one or more transporters, which would further modify the overall transport characteristics. The effect of butyrate on

$\text{Cl}^-$  secretion is of particular clinical interest, since this may reduce colonic secretion in enterotoxin-induced diarrhoea such as cholera.

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