Functional role of bicarbonate in propionate transport across guinea-pig isolated caecum and proximal colon

Wolfgang v. Engelhardt, Gerolf Gros*, Marion Burmester, Kathrin Hansen, Gerhild Becker and Gerhard Rechkemmer

*Departments of Physiology, School of Veterinary Medicine and *School of Medicine, Bischofsholer Damm 15, D-30173 Hannover, Germany*

1. Unidirectional fluxes of propionate across isolated epithelia from the guinea-pig caecum and proximal colon were measured under short-circuit current conditions. In the caecum and proximal colon the serosal-to-mucosal propionate flux (J^{Pr}_{sm}) was higher than mucosal-to-serosal flux (J^{Pr}_{ms}), resulting in a net secretory flux of propionate.

2. HCO_3^-–CO_2-free solution reduced J^{Pr}_{ms} in the caecum and proximal colon markedly; J^{Pr}_{sm} was not (caecum) or little (proximal colon) affected. The subsequent addition of acetazolamide caused a further decrease in J^{Pr}_{ms} in the proximal colon, but not in the caecum.

3. In HCO_3^--containing solutions acetazolamide or ethoxzolamide inhibited J^{Pr}_{ms}; J^{Pr}_{sm} was not affected. A macromolecular carbonic anhydrase inhibitor, prontosil-dextran, had no effect on propionate fluxes, indicating that the intracellular carbonic anhydrase is of importance for short-chain fatty acid transport.

4. Subsequent to carbonic anhydrase inhibition, mucosal addition of amiloride caused a slight further decrease of J^{Pr}_{ms} in the caecum and proximal colon; J^{Pr}_{sm} was not affected.

5. Results support the view that a considerable proportion of short-chain fatty acids (SCFAs) is absorbed via a $SCFA^-$–HCO_3^- exchange.

The total concentration of short-chain fatty acids (SCFAs) in hindgut contents is around 100 mM, of which propionate (Pr) amounts to 10–20 mM (Rechkemmer, Rönnau & Engelhardt, 1988). SCFAs are rapidly absorbed in all segments of the large intestine. Inhibition of the proton antiport systems in the apical membrane indicated that in the caecum of guinea-pig at least 35 %, and in the proximal colon 30–50 %, of SCFAs are absorbed in the lipid-soluble, undissociated form (Engelhardt, Burmester, Hansen, Becker & Rechkemmer, 1993). Most probably the remaining proportion of SCFAs is absorbed in the dissociated form. Transepithelial transport of SCFAs in guinea-pig colon is electroneutral, but in the caecum a small electrogenic component may be present (authors' unpublished observations). Thus, $SCFA^-$ anions have to be absorbed either by electroneutral anion exchange or in cotransport with a cation.

Several earlier *in vivo* studies showed that bicarbonate accumulated in the lumen of the large intestine when SCFAs were absorbed (Engelhardt & Rechkemmer, 1983). Recent studies with apical membrane vesicles of rat distal colon (Mascolo, Rajendran & Binder, 1991) and human colon (Harig, Knaup, Shoshara, Dudeja, Ramaswamy & Brasitus, 1990) and with basolateral membrane vesicles of rat distal colon (Reynolds, Rajendran & Binder, 1991) provided evidence for a $SCFA^-$–HCO_3^- exchange. HCO_3^- could be gained from the carbonic anhydrase-catalysed conversion of metabolically derived CO_2. Carbonic anhydrase activity (CA) is high in the large intestinal mucosa (Carter & Parsons, 1970; Lönnerholm, 1977; Lacy & Colony, 1985; Charney, Wagner, Birnbaum & Johnstone, 1986).

The aim of this study was to investigate the influence of bicarbonate and CA inhibition on unidirectional fluxes of propionate in the caecum and the proximal colon of the guinea-pig. Propionate was chosen from the three major SCFAs since it is not converted to ketone bodies, and metabolism to CO_2 is very small in guinea-pig colonic epithelium (Wirthensohn, 1980).

METHODS

Animals and preparations

Male guinea-pigs (body weight 550–700 g) were fed a pelleted standard diet (Altromin No. 3122, Altromin, Lage, Germany). Water and food were available *ad libitum*. The animals were maintained on a 12 h light:12 h dark photoperiod. They were killed between 8.00 and 9.00 a.m. by decapitation. Caecum and proximal colon were removed, flushed with cold Ringer solution to remove luminal contents and placed into ice-cold Ringer solution gassed continuously with a mixture of 95 % O_2–5 % CO_2. Since prostaglandins are known to influence electrolyte transport across the hindgut epithelium (Halm &

Frizzell, 1986), indomethacin (10^{-6} M) was routinely added to the solutions to inhibit endogenous prostaglandin formation. Indomethacin at this concentration does not have a major influence on unidirectional fluxes of propionate, although short-circuit current and transepithelial conductance are affected significantly in the caecum (Table 1).

The proximal colon (starting from the ampulla caeci) was cut into four 2-cm-long pieces and opened along the mesenteric border. The caecum was cut along the taenia into strips. Muscle layers were manually dissected with forceps. The mucosal sheets were mounted in Ussing chambers with an exposed surface area of 1·13 cm^2. A thin layer of silicon grease (Baysilon; Bayer AG, Leverkusen, Germany) on the chamber diminished edge damage. Four adjacent tissues from the caecum and the proximal colon were mounted from each animal.

Tissues were incubated with 10 ml Ringer solution (37 °C) on both sides. The solution was circulated by a gas lift system using the 95 % O_2–5 % CO_2 mixture or 100 % O_2 in HCO_3^-–CO_2-free solution. The pH of the Ringer solutions ranged from 7·35 to 7·45.

Electrical measurements

Each chamber was connected to an automatic, computer-controlled voltage-clamp amplifier (AC Copy, Aachen, Germany). Fluid resistance was determined before mounting the tissues and automatically corrected during the experiment. Transepithelial potential differences (V_t) were measured with Ringer–agar bridges connected to calomel half-cells and referenced to the mucosal solution. The short-circuit current (I_{sc}) was passed through Ringer–agar bridges connected to Ag–AgCl electrodes in 3 M KCl; I_{sc} was considered positive for cation flow from the mucosal to the serosal side.

Initially, mounted tissues were left under open-circuit conditions for about 30 min. The transepithelial conductance (g_t) was determined each minute by bipolar current pulses of 100 $\mu A\ cm^{-2}$ and 500 ms duration. All electrical parameters (V_t, I_{sc} and g_t) were printed out at intervals of 1 min.

Isotopic measurements

Pairs of tissues of similar g_t from the caecum and the proximal colon were selected to measure mucosal-to-serosal (J_{ms}) and serosal-to-mucosal (J_{sm}) fluxes. All isotope flux experiments were carried out under short-circuit conditions, when 2·5 μCi (92·5 kBq) of [^{14}C]propionate (sodium salt, 1-^{14}C; Du Pont de Nemours, Dreieich, Germany) was added to either the mucosal or the serosal solution. After an equilibrium period of 30 min 0·5 ml aliquots were taken at 10 min intervals for 3–4 h from the solution in which [^{14}C]propionate was not initially added. The sample volume was replaced by an equal volume of unlabelled solution. This was taken into account in the flux calculations.

Solutions

All chemicals were of analytical grade (Merck, Darmstadt, Germany). The standard Ringer solution contained (mM): 140 Na^+, 124 Cl^-, 21 HCO_3^-, 5·4 K^+, 2·4 HPO_4^{2-}, 0·6 $H_2PO_4^-$, 1·2 Mg^{2+}, 1·2 Ca^{2+}, 10 glucose and 10 propionate (Pr). The HCO_3^-–CO_2-free solution contained (mM): 140 Na^+, 124 Cl^-, 5·4 K^+, 2·4 HPO_4^{2-}, 0·6 $H_2PO_4^-$, 1·2 Mg^{2+}, 1·2 Ca^{2+}, 10 glucose and 21 Hepes. Propionate (10 mM) was added as the sodium salt. Osmolarity was adjusted in all solutions to 300 osmol l^{-1} with mannitol. The pH was adjusted to 7·4.

The following drugs were used: amiloride hydrochloride and indomethacin (Sigma, Deisenhofen, Germany), acetazolamide sodium (Lederle, Wolfratshausen, Germany), ethoxzolamide (Merck). Prontosil-dextran (MW 5000) was prepared as described recently (Geers, Gros & Gärtner, 1985).

Statistics.

Results are expressed as means ± S.E.M.; n designates the number of tissues (J_{ms}, J_{sm}) or of paired tissues (J_{net}, I_{sc}, g_t). Differences were estimated using Student's paired or unpaired two-sided t test, as appropriate.

RESULTS

Effect of HCO_3^-–CO_2-free solution on unidirectional fluxes of propionate

When HCO_3^-–CO_2-free solutions were present on both sides of the mucosa of the caecum and the proximal colon J_{ms}^{Pr} was diminished compared to standard Ringer solution (Fig. 1). In the caecum J_{ms}^{Pr} was reduced by 39 %, in the proximal colon by 45 %. No (caecum), or only a small effect (proximal colon reduction of 17 %), was observed for J_{sm}^{Pr}. In the caecum and in the proximal colon g_t did not change significantly when HCO_3^-–CO_2-free solutions were used.

In further experiments using HCO_3^-–CO_2-free solutions carbonic anhydrase activity was inhibited by acetazolamide (Fig. 2). This means that the intracellular generation of HCO_3^- and H^+ from carbon dioxide produced by cellular

Table 1. Unidirectional fluxes in presence of indomethacin

	Caecum				Proximal colon			
	J_{ms}^{Pr}	J_{sm}^{Pr}	g_t	I_{sc}	J_{ms}^{Pr}	J_{sm}^{Pr}	g_t	I_{sc}
Control	0·91 ± 0·04	1·42 ± 0·05	9·2 ± 1·1	3·54 ± 0·57	0·51 ± 0·02	0·77 ± 0·05	13·3 ± 0·8	0·94 ± 0·14
	*	n.s.	***	***	n.s.	n.s.	n.s.	*
Indomethacin	0·87 ± 0·04	1·37 ± 0·05	6·9 ± 0·8	2·54 ± 0·41	0·50 ± 0·02	0·74 ± 0·04	12·3 ± 0·9	0·82 ± 0·13

Unidirectional fluxes of propionate (μequiv $cm^{-2}\ h^{-1}$), transepithelial conductance g_t (ms cm^{-2}) and short-circuit current I_{sc} (μequiv $cm^{-2}\ h^{-1}$) in the absence or presence of indomethacin (10^{-6} M). 10 mM propionate on both sides of the mucosa. Seven epithelia in each group; for J_{sm}^{Pr} in the proximal colon only five. Means ± S.E.M. are given. * $P < 0·05$; *** $P < 0·001$; n.s., not significant.

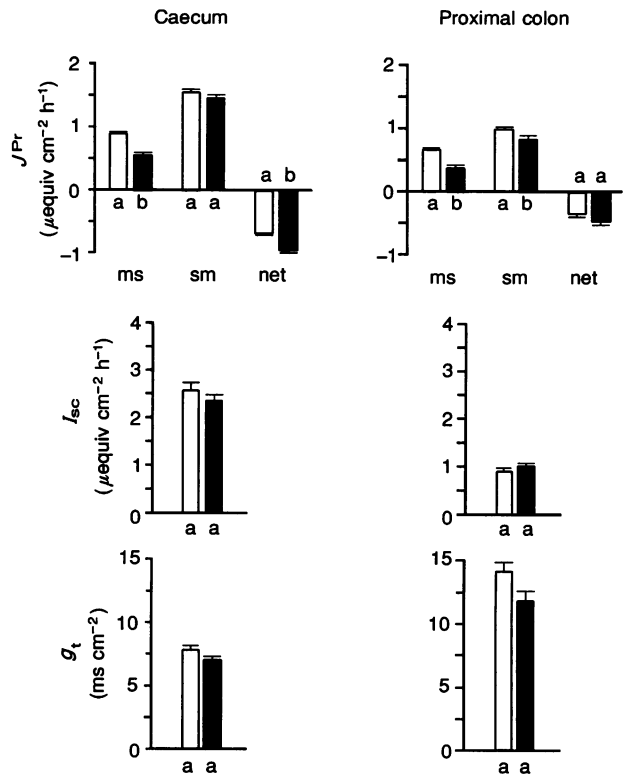

Figure 1. Effects of HCO_3^-–CO_2-free solutions
Effects of HCO_3^-–CO_2-free solutions compared to standard Ringer solution ($n = 10$ or 8 for caecum and proximal colon, respectively) on mucosal-to-serosal (ms) and serosal-to-mucosal (sm) and net fluxes of propionate, short-circuit current (I_{sc}) and transepithelial conductance (g_t). 10 mM propionate was present on both sides of the mucosa. Means ± S.E.M. are given. □, control solution with 10mM propionate. ■, HCO_3^-–CO_2-free solution with 10 mM propionate. Bars with the same subscripts are not significantly different ($P < 0.05$).

metabolism is slowed down. Addition of acetazolamide had no effect on J^{Pr}_{ms} in the caecum, but acetazolamide caused a 39 % decrease in the proximal colon; subsequent addition of amiloride had no further effect in either the caecum or the proximal colon. Acetazolamide decreased g_t slightly in both. It diminished I_{sc} in the caecum, but had no effect on I_{sc} in the proximal colon.

Effects of carbonic anhydrase inhibition on unidirectional fluxes

In the caecum and less markedly in the proximal colon J^{Pr}_{ms} was significantly lower than J^{Pr}_{sm}, resulting in a net propionate secretion (Fig. 3). Intracellular generation of HCO_3^- and H^+ was diminished in these studies with

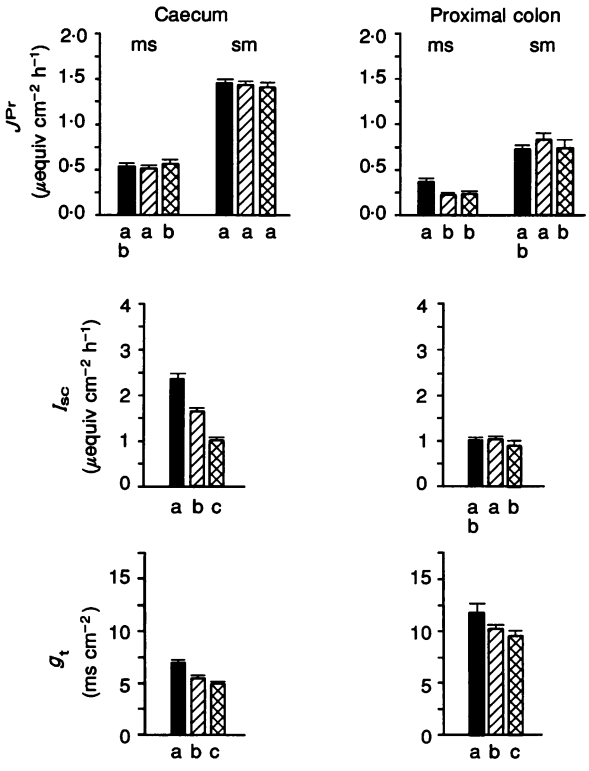

Figure 2. Effects of acetazolamide and amiloride under HCO_3^-–CO_2-free conditions
Unidirectional fluxes of propionate under HCO_3^-–CO_2-free conditions. Propionate (10 mM) was present on both sides ($n = 8$–10) of the mucosa. Acetazolamide (10^{-3} M) was present in both mucosal and serosal solutions, subsequently amiloride (10^{-3} M) was added to the mucosal solution. Means ± S.E.M. are given. ■, control, HCO_3^-–CO_2-free solution with 10 mM propionate. ▨, acetazolamide (10^{-3} M) added to mucosal and serosal solutions. ▩, amiloride (10^{-3} M) added to mucosal solution only. Bars with the same subscripts are not significantly different ($P < 0.05$).

Table 2. Unidirectional fluxes in the absence and presence of the carbonic anhydrase inhibitors

	Caecum			Proximal colon		
	J^{Pr}_{ms}	J^{Pr}_{sm}	J^{Pr}_{net}	J^{Pr}_{ms}	J^{Pr}_{sm}	J^{Pr}_{net}
Control	0·87 ± 0·07	1·65 ± 0·12	−0·78 ± 0·09	0·69 ± 0·05	1·01 ± 0·05	−0·32 ± 0·09
	**	n.s.	***	**	*	**
Ethoxzolamide	0·54 ± 0·04	1·70 ± 0·11	−1·16 ± 0·09	0·45 ± 0·07	1·10 ± 0·07	−0·65 ± 1·02
Control	0·88 ± 0·08	1·67 ± 0·16	−0·79 ± 0·10	0·80 ± 0·06	0·95 ± 0·07	−0·15 ± 0·06
	**	n.s.	**	**	n.s.	**
Acetazolamide	0·43 ± 0·05	1·80 ± 0·19	−1·37 ± 0·16	0·55 ± 0·06	1·02 ± 0·08	−0·47 ± 0·09
Statistical significance						
between controls	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
between ethox. and acet.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Unidirectional fluxes of propionate (μequiv cm^{-2} h^{-1}) in the absence and presence of the carbonic anhydrase inhibitors ethoxzolamide (ethox., 10^{-5} M) and acetazolamide (acet., 10^{-3} M). Usually five epithelia in each group; for acetazolamide in the caecum only four. 10 mM propionate on both sides of the mucosa. Means ± S.E.M. are given.* $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$; n.s., not significant.

HCO_3^-–CO_2-containing solutions by inhibition of CA. Addition of either ethoxzolamide (10^{-5} M) or acetazolamide (10^{-3} M) to both sides of the epithelium decreased J^{Pr}_{ms} in the caecum by 44 % and in the proximal colon by 33 %. This inhibition was quantitatively similar with either ethoxzolamide or acetazolamide (Table 2); the data from both experiments were therefore combined in Fig. 3. Subsequent addition of amiloride to the mucosal solution further decreased J^{Pr}_{ms} by about 20 % in the caecum and 16 % in the proximal colon. The total inhibition of J^{Pr}_{ms} by acetazolamide or ethoxzolamide and mucosal amiloride in the caecum and in the proximal colon was similar to the values reported recently for amiloride alone (Engelhardt *et al.* 1993).

Carbonic anhydrase inhibition reduced I_{sc} in the caecum (−23 %), but in the proximal colon it was increased slightly (Fig. 3). In the caecum carbonic anhydrase inhibition had no

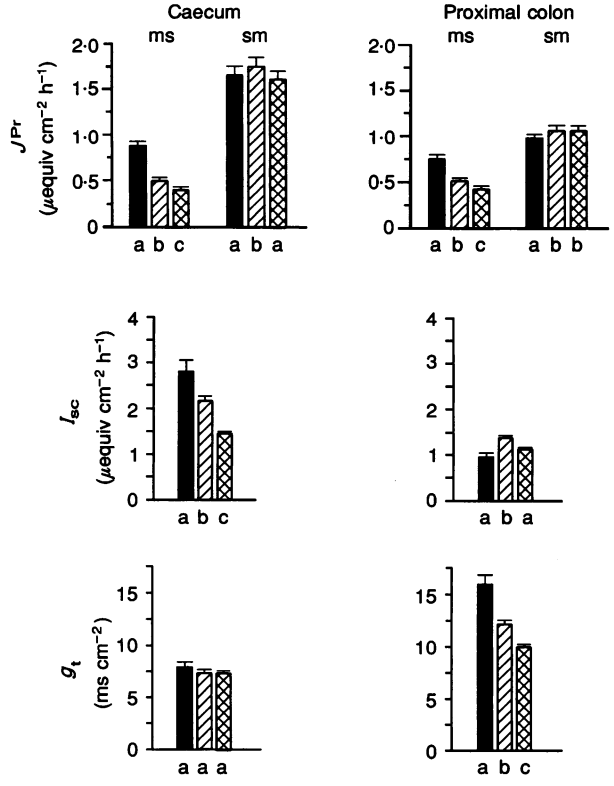

Figure 3. Effects of carbonic anhydrase inhibition
Unidirectional fluxes of propionate before and after carbonic anhydrase inhibition ($n = 9$–10). Means ± S.E.M. are given. ■, control Ringer solution with 10 mM propionate in the presence of HCO_3^- and CO_2. ▨, carbonic anhydrase inhibition with ethoxzolamide (10^{-5} M) or acetazolamide (10^{-3} M) on both sides of the mucosa. ▩, subsequently apical Na^+–H^+ exchange was inhibited with amiloride (10^{-3} M,) when added to the mucosal solution. Effects on short-circuit current (I_{sc}) and transepithelial conductance (g_t) are given. Bars with the same subscripts are not significantly different ($P < 0·05$).

effect and in the proximal colon g_t was slightly diminished. Addition of amiloride did not change g_t in the caecum, but decreased it in the proximal colon.

Cellular localization of the carbonic anhydrase involved in SCFA transport

To obtain information on the localization of carbonic anhydrase involved in SCFA transport a macromolecular carbonic anhydrase inhibitor, prontosil-dextran (Geers *et al.* 1985), was used, and J^{Pr}_{ms} and J^{Pr}_{sm} were measured. Prontosil (10^{-4} M)-dextran (MW 5000) was added to the solutions of both sides. As a control, fluxes were estimated in epithelia where dextran alone had been added. Prontosil-dextran had no systematic influence on J^{Pr}_{ms} or J^{Pr}_{sm} compared to the control with dextran (Table 3). This indicates that the carbonic anhydrase catalysing hydration of carbon dioxide and providing HCO_3^- and/or H^+ for J^{Pr}_{ms} is mainly intracellularly located. However, findings with dextran and prontosil-dextran are less homogeneous than those from the earlier experiments. Therefore a minor effect of the extracellular CA cannot be totally excluded.

DISCUSSION

Evidence for an apical $SCFA^-$–HCO_3^- exchange

From earlier *in vitro* (Engelhardt & Rechkemmer, 1992; Engelhardt *et al.* 1993) and *in situ* studies (Luciano, Reale, Rechkemmer & Engelhardt, 1984) we concluded that in caecum and proximal colon of guinea-pig 35–50 % of SCFA absorption may occur in the protonated form. It was assumed that in addition to such non-ionic diffusion SCFAs are also transported in the ionized form, most probably by a $SCFA^-$–HCO_3^- exchange. This is confirmed by the data presented showing that in HCO_3^-–CO_2-free solutions and after CA inhibition in HCO_3^--containing solutions J^{Pr}_{ms} was significantly reduced (Figs 1 and 3). In HCO_3^-–CO_2-free solutions some HCO_3^- may still be available from the carbonic anhydrase-catalysed conversion of CO_2 from cell metabolism. To minimize such intracellular generation of HCO_3^- the intracellular carbonic anhydrase was inhibited in these experiments with HCO_3^--free solutions. As a result in the proximal colon (not in the caecum) J^{Pr}_{ms} was further diminished (Fig. 2). These findings strongly support the view that a considerable proportion of short-chain fatty acids is absorbed via $SCFA^-$–HCO_3^- exchange.

The marked inhibition of J^{Pr}_{ms} in HCO_3^-–CO_2-free solutions in our study is contradictory to findings in the rabbit proximal colon (Sellin & DeSoignie, 1990) where no differences in unidirectional fluxes of propionate were observed when comparing bicarbonate-containing and bicarbonate-free solutions at pH 7·4; at pH 6·8 fluxes were even increased in bicarbonate-free solutions. We have no explanation for these apparent genus differences. Experiments with apical membrane vesicles of rat distal colon (Mascolo *et al.* 1991), human colon (Harig *et al.* 1990) and basolateral membrane vesicles of rat distal colon (Reynolds *et al.* 1991) also indicated the presence of a bicarbonate-dependent SCFA anion uptake. However, this uptake was not inhibited by amiloride and acetazolamide (Mascolo *et al.* 1991). In *Tilapia*, a herbivorous fish, an electroneutral, one-for-one antiport of acetate with bicarbonate across the apical and the basolateral membrane was shown (Titus & Ahearn, 1988, 1991).

The H^+ for the proton antiport systems may be derived from CO_2 conversion within the cell

Proton-secreting systems in the apical membrane supply protons for the mucosal-to-serosal non-ionic diffusion of SCFAs. Inhibition of the apical Na^+–H^+ exchange by adding amiloride to the mucosal solution diminished J^{SCFA}_{ms} in the caecum and in the proximal colon of guinea-pig (Engelhardt *et al.* 1993). Carbonic anhydrase inhibition alone (Fig. 3) led to a lower reduction in J^{Pr}_{ms} than in our recent studies where the proton antiport systems were blocked. After adding amiloride to the mucosal solution on top of the carbonic anhydrase inhibition, J^{Pr}_{ms} in the present study was diminished in the caecum and proximal colon to an extent similar to that in these recent studies. This indicates that CA inhibition diminished both the availability of protons for the Na^+–H^+ exchange in the apical membrane and thus for the transport of SCFAs in the undissociated form, as well as the intracellular HCO_3^- supply for the transport of SCFAs in the ionized form.

Table 3. Unidirectional fluxes of propionate

	Caecum				Proximal colon			
	J^{Pr}_{ms}	n	J^{Pr}_{sm}	n	J^{Pr}_{ms}	n	J^{Pr}_{sm}	n
Control	0·74 ± 0·09	6	1·27 ± 0·14	6	0·57 ± 0·08	6	0·78 ± 0·09	6
	n.s.		n.s.		n.s.		n.s.	
Prontosil-dextran	0·68 ± 0·07	4	1·26 ± 0·04	7	0·46 ± 0·03	6	0·70 ± 0·05	6

Unidirectional fluxes of propionate (μequiv m^{-2} h^{-1}) with prontosil (10^{-4} M)-dextran or equimolar amounts of dextran (control) on both sides of the epithelium. 10 mM propionate on both sides. Means ± S.E.M. are given; n, number of epithelia; n.s., not significant.

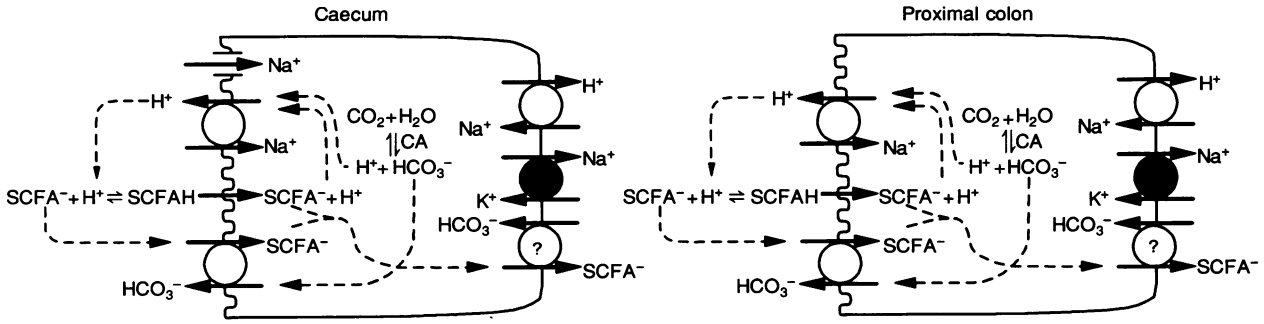

Figure 4. Model for the absorption of short-chain fatty acids in the caecum and the proximal colon of guinea-pig

Effects of carbonic anhydrase inhibition on transepithelial conductance and short-circuit current.

Carbonic anhydrase inhibition did not change g_t in the caecum but decreased it in the proximal colon by about 20 % (Fig. 3). However, CA inhibition increased g_t in the distal colon of guinea-pig nearly threefold (data not given). We have no explanation for this selective extreme increase in g_t in the distal colon of guinea-pig. Likewise the effect of CA inhibition on I_{sc} in the caecum is not totally clear. CA inhibitors seem to have multiple effects on transport systems, especially in the caecum. Acetazolamide, as well as ethoxzolamide, markedly diminished both J^{Na}_{ms} and I_{sc} in the caecum and to a lesser extent J^{Na}_{ms} in the proximal colon of guinea-pig (authors' unpublished observation and Fig. 3). In the rabbit caecum most of the sodium ions enter the cell via a special type of Na^+ channel (Sellin, Oyarzabal & Cragoe, 1988); this entry is insensitive to low doses of amiloride but can be blocked by the amiloride analogue phenamil. Similarly, in the guinea-pig epithelium an apical membrane Na^+ channel with a rather low sensitivity to inhibition by amiloride exists (Rechkemmer & Engelhardt, 1993). It is not clear how the CA inhibitors used in our studies affect the Na^+ transport in the apical membrane of the caecum.

Localization of the carbonic anhydrase involved in $SCFA^-$ transport

The decrease of J^{Pr}_{ms} by CA inhibition appears to act mainly by an intracellular inhibition. Ethoxzolamide is supposed to enter the enterocytes easily. The observation that acetazolamide produces essentially the same effects but at a concentration 100 times higher than ethoxzolamide suggests that lipid solubility is important for the observed CA inhibitory effect. This is supported by experiments with the macromolecular carbonic anhydrase inhibitor prontosil-dextran which showed no significant effect on $SCFA^-$ transport.

Model for $SCFAH/SCFA^-$ absorption in the caecum and the proximal colon of guinea-pig

Most of the SCFAs (95–99 %) are present as anions at the physiological pH of the large intestinal contents. From inhibitory studies we concluded that ~35 and 40–50 % of the mucosal-to-serosal SCFA transport in caecum and proximal colon of guinea-pig respectively is due to non-ionic diffusion (Engelhardt *et al.* 1993). The H^+-secreting systems in the apical membrane in the caecum and the proximal colon of guinea-pig appeared to be from Na^+–H^+ exchange (Fig. 4). The present findings and recent vesicle studies indicate that the remaining mucosal-to-serosal SCFA transport results from an $SCFA^-$ anion exchange with HCO_3^-. The intracellular carbonic anhydrase catalyses conversion of CO_2 to HCO_3^- and H^+. Intracellular HCO_3^- is made available for $SCFA^-$–HCO_3^- exchange, and H^+ for Na^+–H^+ exchange in the apical membrane and thus provides protons for non-ionic diffusion of SCFAs. Mechanisms responsible for the exit of SCFAs across the basolateral membrane are not well understood; bicarbonate seems to be involved.

REFERENCES

Carter, M. J. & Parsons, D. S. (1970). The carbonic anhydrase of some guinea-pig tissues. *Biochimica et Biophysica Acta* **206**, 190–192.

Charney, A. N., Wagner, J. D., Birnbaum, G. J. & Johnstone, J. N. (1986). Functional role of carbonic anhydrase in intestinal electrolyte transport. *American Journal of Physiology* **251**, G682–687.

Engelhardt, W. v., 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. *Journal of Physiology* **460**, 455–466.

Engelhardt, W. v. & Rechkemmer, G. (1983). Absorption of inorganic ions and short-chain fatty acids in the colon of mammals. In *Intestinal Transport*, ed. Gilles-Baillien, M. & Gilles, R., pp. 26–45. Springer-Verlag, Heidelberg.

ENGELHARDT, W. v. & RECHKEMMER, G. (1992). Segmental differences of short-chain fatty acid transport across guinea-pig large intestine. *Experimental Physiology* **77**, 491–499.

GEERS, C., GROS, G. & GÄRTNER, A. (1985). Extracellular carbonic anhydrase of skeletal muscle associated with the sarcolemma. *Journal of Applied Physiology* **59**, 548–558.

HALM, D. R. & FRIZZELL, R. A. (1986). Active K transport across rabbit distal colon: relation to Na absorption and Cl secretion. *American Journal of Physiology* **251**, C252–267.

HARIG, J. M., KNAUP, S. M., SHOSHARA, J., DUDEJA, P. K., RAMASWAMY, K. & BRASITUS, T. A. (1990). Transport of *n*-butyrate into human colonic luminal membrane vesicles. *Gastroenterology* **98**, A543.

LACY, E. R. & COLONY, P. C. (1985). Localization of carbonic anhydrase activity in the developing rat colon. *Gastroenterology* **89**, 138–150.

LÖNNERHOLM, G. (1977). Carbonic anhydrase in the intestinal tract of the guinea-pig. *Acta Physiologica Scandinavica* **99**, 53–61.

LUCIANO, L., REALE, E., RECHKEMMER, G. & ENGELHARDT, W. v. (1984). Structure of zonulae occludentes and the permeability of the epithelium to short-chain fatty acids in the proximal and the distal colon of guinea-pig. *Journal of Membrane Biology* **82**, 145–156.

MASCOLO, N., RAJENDRAN, V. M. & BINDER, H. J. (1991). Mechanism of short-chain fatty acid uptake by apical membrane vesicles of rat distal colon. *Gastroenterology* **101**, 331–338.

RECHKEMMER, G., RÖNNAU, K. & ENGELHARDT, W. v. (1988). Fermentation of polysaccharides and absorption of short chain fatty acids in the mammalian hindgut. *Comparative Biochemistry and Physiology* A **90**, 563–568.

RECHKEMMER, G. & ENGELHARDT, W. v. (1993). Absorption and secretion of electrolytes and short-chain fatty acids in the guinea-pig large intestine. In *Ion Transport in Vertebrate Colon*, ed. CLAUSS, W., *Advances in Comparative and Environmental Physiology*, vol. 16, pp. 139–167. Springer, Heidelberg, New York.

REYNOLDS, D., RAJENDRAN, V. M. & BINDER, H. J. (1991). Butyrate transport in rat distal colon basolateral membrane vesicles: Evidence for bicarbonate/butyrate exchange. *Gastroenterology* **100**, A838.

SELLIN, J. H., & DESOIGNIE, R. (1990). Short-chain fatty acid absorption in rabbit colon *in vitro*. *Gastroenterology* **99**, 676–683.

SELLIN, J. H., OYARZABAL, H. & CRAGOE, E. J. (1988). Electrogenic sodium absorption in rabbit cecum *in vitro*. *Journal of Clinical Investigation* **81**, 1275–1283.

TITUS, E. & AHEARN, G. A. (1988). Short-chain fatty acid transport in the intestine of a herbivorous teleost. *Journal of Experimental Biology* **135**, 77–94.

TITUS, E. & AHEARN, G. A. (1991). Transintestinal acetate transport in a herbivorous teleost: anion exchange at the basolateral membrane. *Journal of Experimental Biology* **156**, 41–61.

WIRTHENSOHN, K. (1980). Der Stoffwechsel kurzkettiger Fettsäuren im Colonepithel des Meerschweinchens und seine Bedeutung für die Natriumresorption. PhD Thesis, Stuttgart-Hohenheim University, Stuttgart.

Acknowledgements

The project was supported by a grant from the Deutsche Forschungsgemeinschaft, SFB 280 'Gastrointestinale Barriere'.

Received 22 February 1993; accepted 26 October 1993.