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EFFECTS OF AMILORIDE AND OUABAIN ON SHORT-CHAIN FATTY ACID TRANSPORT IN GUINEA-PIG LARGE INTESTINE

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SUMMARY

1. Effects of H^+ secreting mechanisms on unidirectional passage of the short-chain fatty acids (SCFA) acetate, propionate and *n*-butyrate across isolated guinea-pig caecum, proximal and distal colon were studied under short-circuit current conditions in Ussing chamber isotope flux experiments.

2. In the caecum and the proximal colon the serosal-to-mucosal fluxes (J_{sm}) were higher than the mucosal-to-serosal fluxes (J_{ms}). Thus a net secretion of SCFA was present in the caecum and proximal colon. The higher J_{sm} appears to be coupled to the Na^+ gradient established by the basolateral membrane Na^+–K^+-ATPase, whereas J_{ms} is related to the operation of an apical membrane Na^+–H^+ exchanger. Inhibition of Na^+–H^+ exchange by amiloride (1 mM) added to the mucosal solution decreased J_{ms} of SCFA in caecum and in proximal colon, but had no major effect in distal colon.

3. In distal colon J_{ms} exceeds J_{sm} and thus a net absorption of SCFA was observed. J_{ms} is Na^+ independent and coupled to the activity of the apical membrane K^+–H^+-ATPase. Inhibition of the K^+–H^+-ATPase by addition of ouabain (0·1 mM) to the mucosal solution diminished J_{ms} in the distal colon; in the caecum and proximal colon ouabain had no effect on J_{ms}.

4. Neither amiloride nor ouabain caused major changes in J_{sm} in any of the large intestinal segments.

5. In conclusion, absorption of SCFA, e.g. J_{ms}, in all large intestinal segments is related to the presence and activity of H^+ secreting systems located in the apical membrane of colonocytes. In the caecum and proximal colon the predominant system appears to be Na^+–H^+ exchange, and in the distal colon K^+–H^+-ATPase. Supply of H^+ ions allows protonation of SCFA anions and subsequent permeation by non-ionic diffusion across the apical membrane. These mechanisms account for 35, 40–50 and 60–80% of SCFA transport in the caecum, the proximal and the distal colon of guinea-pig, respectively. The nature of the Na^+-dependent secretory pathway in the caecum and proximal colon remains to be determined.

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INTRODUCTION

Short-chain fatty acids (SCFA), primarily acetate, propionate and *n*-butyrate, are produced in the large intestine of mammals by anaerobic microbial fermentation of undigested carbohydrates. Due to continuous production SCFA concentrations are about 100 mM in hindgut contents (Rechkemmer, Rönnau & Engelhardt, 1988). The pK_a of these weak monocarboxylic acids is around 4·8 and thus approximately 99% of SCFA are present as anions at the physiological pH of hindgut digesta. Until recently it was assumed that a major portion of short-chain fatty acids is absorbed in the large intestine in the lipid-soluble, undissociated form (Luciano, Reale, Rechkemmer & Engelhardt, 1984; Rönnau, Guth & Engelhardt, 1989; Engelhardt & Rechkemmer, 1992). This idea has been challenged by recent uptake studies with apical membrane vesicles from rat and human colon suggesting the involvement of anion exchange systems in the transmembrane transport of SCFA (Harig, Knaup, Shoshara, Dudeja, Ramaswamy & Brasitus, 1990; Mascolo, Rajendran & Binder, 1991).

If non-ionic diffusion across the hindgut epithelium would be a major factor determining SCFA transport, SCFA anions have to become protonated. Thus, the availability of H^+ ions could limit the absorption of SCFA. H^+ ions may be gained at the luminal side of the apical membrane by: (1) amiloride-sensitive Na^+–H^+ exchange, occurring in the caecum and the proximal colon of guinea-pigs (Rechkemmer, 1988), and (2) K^+–H^+-ATPase present in the apical membrane of the distal colon of guinea-pigs (Suzuki & Kaneko, 1987, 1989; Watanabe, Suzuki & Suzuki, 1990). The present study was designed to evaluate the role of H^+ antiport systems for absorption of SCFA in the different segments of the large intestine in guinea-pigs by specifically inhibiting these systems. Furthermore, in an attempt to characterize the differences in unidirectional SCFA fluxes, experiments with equal concentrations of SCFA on both sides of the mucosa or with only mucosal addition of SCFA, closely resembling the physiological situation, were conducted. The results of this study confirm the importance of the H^+ antiport systems for SCFA transport in the large intestine.

METHODS

Animals and preparation

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 12 h light: 12 h dark photoperiod. They were killed between 08.00 and 09.00 h by decapitation. Caecum, proximal and distal colon were removed, flushed with cold Ringer solution to eliminate luminal contents and placed into ice-cold Ringer solution gassed continuously with a mixture of 95% O_2 and 5% CO_2. Since prostaglandins are known to influence electrolyte transport across the hindgut epithelium (Halm & Frizzell, 1986; Rechkemmer, 1988), indomethacin (10^{-6} M) was routinely added to the solutions to inhibit endogenous prostaglandin formation.

The proximal colon (starting from the ampulla caeci) and the distal colon (from the rectum) were cut into four approximately 2-cm-long pieces and opened along the mesenteric border. The caecum was cut into strips along the taenia. Muscle layers were manually dissected with forceps (Frizzell, Koch & Schultz, 1976). The mucosal sheets were mounted in Ussing chambers with an exposed surface area of 1·13 cm^2 for caecum and proximal colon, and 0·50 cm^2 for the distal colon. A thin layer of silicone grease (Baysilon, Bayer AG, Leverkusen, Germany) on the chamber reduced edge damage. Holding of the sheet by six small stainless-steel needles facilitated a uniform stretching

of the epithelium. Four adjacent tissues from the caecum, the proximal and the distal colon were mounted from each animal.

Tissues were bathed with 10 ml of Ringer solution at 37 °C on both sides. The solution was circulated by a gas lift system using the 95% O_2 and 5% CO_2 mixture. The pH of the Ringer solution ranged from 7·35 to 7·45.

Electrical measurements

Each chamber was connected to an automatic, computer controlled, voltage clamp amplifier (AC Copy, Aachen). Fluid resistance was determined before mounting and automatically corrected for during the experiment. Transepithelial potential differences (V_t) were measured with Ringer–agar bridges connected to calomel half-cells with reference 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 when cations flowed from mucosal to 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 μA cm^{-2} and 500 ms duration. All electrical parameters (V_t, I_{sc} and g_t) were printed out in intervals of 1 min.

Isotopic measurements

Pairs of tissues of similar g_t from the caecum, the proximal colon and the distal colon respectively, were selected to measure mucosal-to-serosal (J_{ms}) and the serosal-to-mucosal (J_{sm}) fluxes. All isotope experiments were carried out under short-circuit current conditions. 2·5 μCi (92·5 kBq) of the appropriate [^{14}C]SCFA (sodium salt, I-^{14}C, from Amersham Buchler, Braunschweig, or Du Pont de Nemours, Dreieich) were added to either the mucosal or serosal solution; 10 mM SCFA was present in both mucosal and serosal solutions. After an equilibrium period of 30 min, 0·5 ml aliquots were taken at 10 min intervals for 3–4 hours from the solution in which [^{14}C]SCFA was not supplemented. The sample volume was replaced by an equal volume of the 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+} and 10 glucose. Sodium salts (10 mM) of acetate (Ac), propionate (Pr) or butyrate (Bu) were added. In the propionate-free solution 10 mM gluconate was used. In the Na^+-free solution, NaCl was replaced with *N*-methyl-D-glucamine chloride; $NaHCO_3$ was substituted by choline bicarbonate. Osmolality was adjusted in all solutions to 300 mosm l^{-1} with mannitol. The following drugs were used: amiloride hydrochloride, ouabain and indomethacin (Sigma, Deisenhofen).

Statistics

Results are expressed as means ± S.E.M. *n* designates the numbers 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-tailed *t* test, as appropriate.

RESULTS

Effects of amiloride and ouabain on unidirectional fluxes of SCFA

Under control conditions, e.g. indomethacin-treated tissues, J_{sm} exceeded J_{ms} for all three SCFA in the caecum and proximal colon, resulting in net SCFA secretion (Fig. 1). Amiloride (10^{-3} M) added to the luminal solution diminished J_{ms} for all three SCFA in the caecum and in the proximal colon by 30–40% (Fig. 1). Except for a small decrease of J_{sm}^{Ac} in the proximal colon, amiloride did not affect J_{sm} in all segments of the large intestine. Thus as a result of the decrease in J_{ms}, addition of amiloride to the luminal solution increased the net secretory fluxes in the caecum and in the proximal colon.

In the distal colon J_{ms} was larger than J_{sm}, and thus a net absorptive flux of SCFA

(Bu > Pr > Ac) was observed (Fig. 1). Amiloride (1 mM) caused a minor, less than 10%, decrease of J_{ms}^{Pr} and J_{ms}^{Bu} ($P < 0.05$) and since J_{sm} for all three SCFA was unchanged, a small decrease in J_{net} of propionate and butyrate was apparent.

Ouabain (0·1 mM) added subsequent to amiloride to the mucosal solution to inhibit the K^+–H^+-ATPase had no marked effects on unidirectional fluxes in the proximal

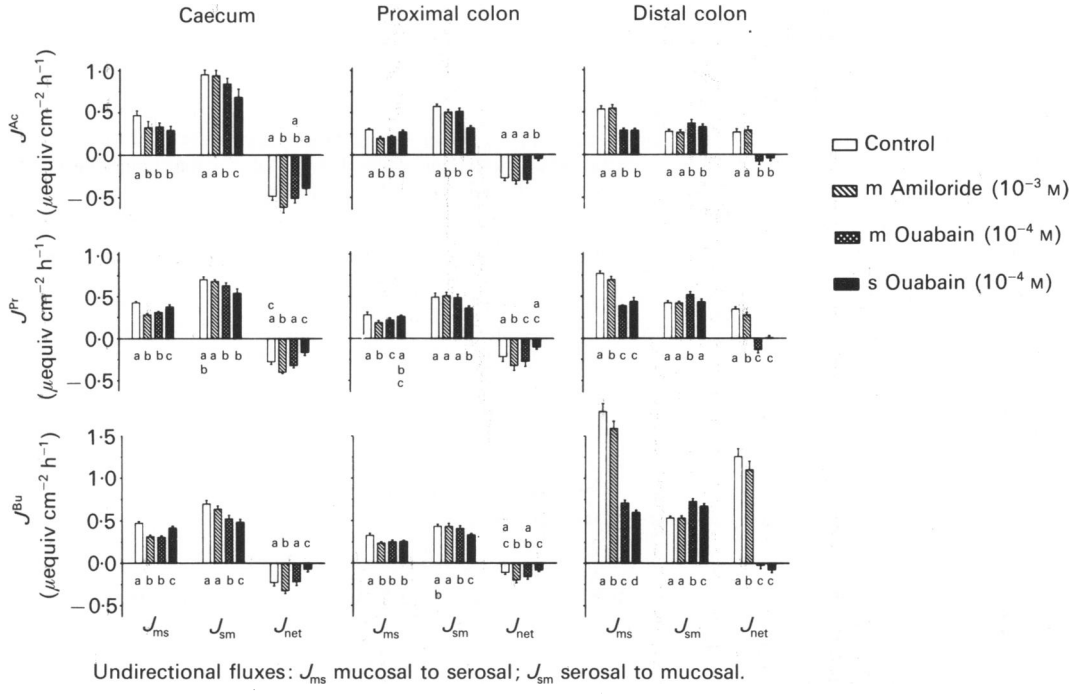

Fig. 1. Effects of amiloride and ouabain added to the mucosal (m) or the serosal (s) solutions on unidirectional fluxes (J) of acetic acid (Ac), propionic acid (Pr) and butyric acid (Br) in guinea-pig caecum, proximal and distal colon. Mean values for unidirectional fluxes are calculated from five to nine epithelia. S.E.M. are expressed as vertical lines. Bars with the same letters (a, b, c and d) are not significantly different ($P < 0.05$).

colon. In the caecum J_{ms} was not altered, but J_{sm} was slightly decreased, leading to a decrease in net secretion of propionic and butyric acid, but not of acetic acid. In the distal colon J_{ms} was highly significantly reduced by 50–60%, and in parallel J_{sm} was increased by about 30%. J_{sm} and J_{ms} became about equal and J_{net} was not different from zero for butyrate. For acetate and propionate J_{sm} even exceeded J_{ms} resulting in a small net secretion in the distal colon.

Finally, ouabain (0·1 mM) was added to the serosal solution in the last part of these experiments to inhibit the basolateral Na^+–K^+-ATPase and transport processes which are coupled to the Na^+ gradient established by the pump. In the caecum and in the proximal colon J_{ms} mostly increased slightly and J_{sm} decreased modestly. In the distal colon J_{sm} decreased slightly. Except for J_{net}^{Ac} in the caecum, net fluxes for the other SCFA in all three large intestinal segments were small and in most cases not significantly different from zero (Fig. 1).

Effects of amiloride and ouabain on electrical parameters

No systematic differences in short-circuit current (I_{sc}) and in transepithelial conductance (g_t) were seen in the experiments employing the three different SCFA. Therefore data for the Ac, Pr and Bu experiments were combined (Fig. 2). Mucosal

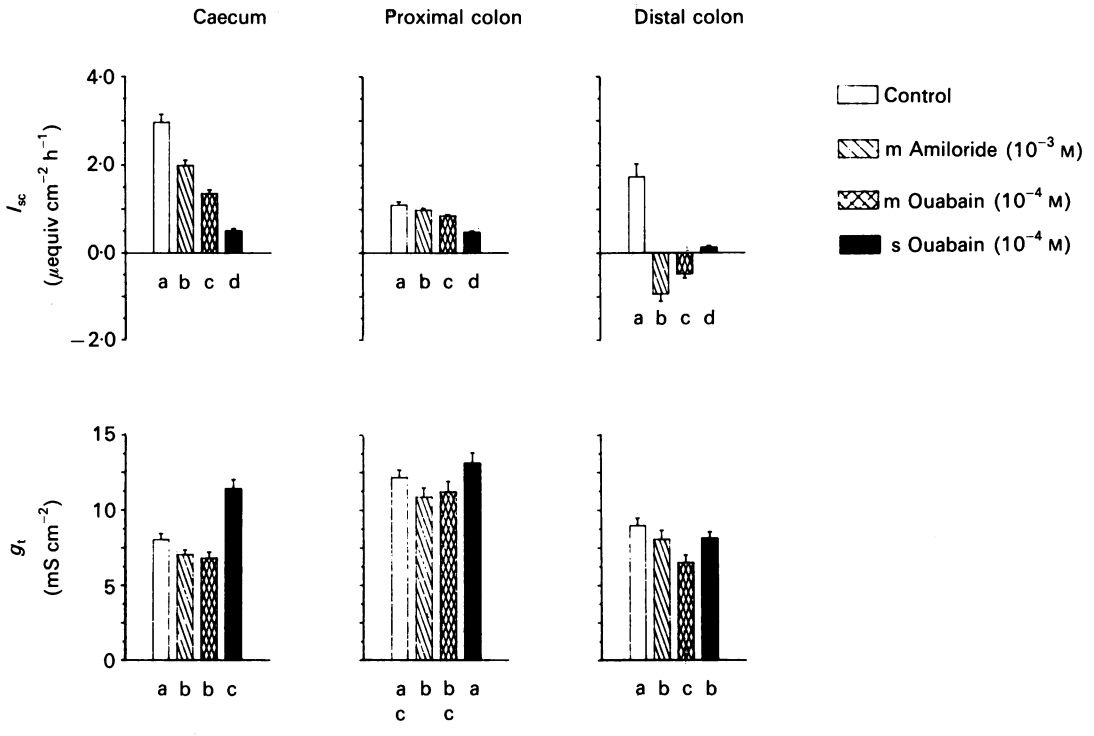

Fig. 2. Effects of amiloride and ouabain added to the mucosal (m) or the serosal solutions on short-circuit current (I_{sc}) and transepithelial conductance (g_t) in guinea-pig caecum, proximal and distal colon. Data are combined from experiments shown in Fig. 1 for all three short-chain fatty acids. Means ± S.E.M. are calculated from nineteen to twenty-one pairs of epithelia. Bars with the same letters (a, b, c and d) are not significantly different ($P < 0.05$).

amiloride (1 mM) decreased I_{sc} in the caecum by 33% and had only a minor effect in the proximal colon. Amiloride had the most pronounced effect in the distal colon; I_{sc} reversed from $+1.8$ to -0.9 μequiv cm^{-2} h^{-1}.

Addition of ouabain to the mucosal solution decreased I_{sc} in the caecum to half the control values; in the proximal colon only a minor decrease was measured. In the distal colon after addition of amiloride I_{sc} became negative; the subsequent supplementation of ouabain to the mucosal solution decreased these values by 50%. The addition of ouabain to the serosal bath led to low I_{sc} values of ~ 0.5 μequiv cm^{-2} h^{-1} in the caecum and proximal colon; I_{sc} in the distal colon was not significantly different from zero.

During the control period g_t was similar in the caecum and in the distal colon ($8 \pm 0{\cdot}4$ and $9 \pm 0{\cdot}5$ mS cm^{-2}). In the proximal colon values were higher ($12{\cdot}2 \pm 0{\cdot}5$ mS cm^{-2}). After amiloride g_t decreased significantly in all three segments. The addition of ouabain to the mucosal solution caused an additional slight decrease

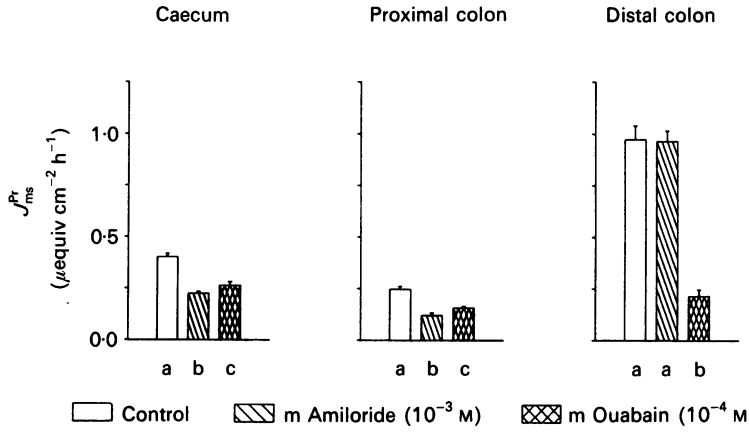

Fig. 3. Effect of proton antiport inhibition on mucosal-to-serosal fluxes of propionate/propionic acid (Pr) when propionate (10 mM) was present in the mucosal solution only. In the serosal solution Pr was substituted with 10 mM gluconate. Amiloride (10^{-3} M) and ouabain (10^{-4} M) were added to the mucosal solution. Means ± S.E.M. were calculated from six to seven epithelia. Bars with the same letters (a, b and c) are not significantly different ($P < 0{\cdot}05$).

in g_t in the distal colon, while in the caecum and proximal colon g_t was not affected by mucosal ouabain. Additional application of ouabain to the serosal side increased g_t significantly in all large intestinal segments. In the caecum significantly higher values than under control conditions were seen. In the proximal colon and in the distal colon values near to control values were observed.

Effects of amiloride and ouabain on J^{Pr}_{ms} when propionate was present in the mucosal solution only

Under physiological conditions SCFA are present in the luminal contents at high concentrations, exceeding 100 mM, while concentrations are below 1 mM in the blood. Under the symmetrical *in vitro* conditions an unphysiologically high J^{SCFA}_{sm} could supply H^+ ions to the mucosal side of the epithelium, and this in turn could affect J^{SCFA}_{ms}. Therefore in a series of experiments propionate (10 mM) was added to the mucosal solution only.

In the SCFA-free serosal solution propionate was substituted by gluconate (asymmetrical condition) (Fig. 3). J^{Pr}_{ms} were similar to the values under symmetrical conditions in all segments (Fig. 1). Inhibition of the Na^+–H^+ exchange in the caecum and proximal colon with amiloride (1 mM) reduced J^{Pr}_{ms} by 34 and 51 %, respectively, and J^{Pr}_{ms} was unaffected in the distal colon. Inhibition of K^+–H^+-ATPase by mucosal ouabain (0·1 mM) diminished J^{Pr}_{ms} in the distal colon highly significantly by 78 %. Only a minor increase in J^{Pr}_{ms} was seen in the caecum and proximal colon.

Effects of Na^+-free solutions on unidirectional fluxes of propionate

J^{Pr}_{ms} and J^{Pr}_{sm} were measured with a conventional Ringer solution (control) or with a Na^+-free solution on both sides of the epithelium (Fig. 4). Similar to the data presented in Fig. 1 in the caecum and proximal colon, J^{Pr}_{sm} was considerably higher

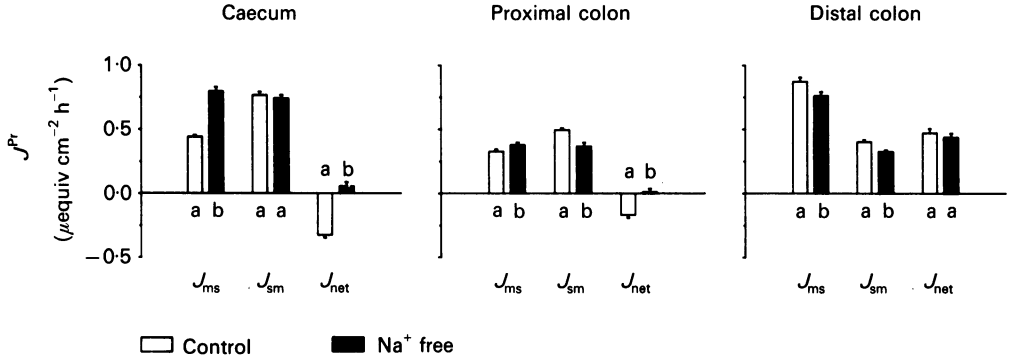

Fig. 4. Comparison of unidirectional and net fluxes of propionate under control and Na^+-free conditions in the caecum, proximal and distal colon. Na^+ was replaced by *N*-methyl-D-glucamine and choline in the Na^+-free solution. Means ± S.E.M. from twenty and twelve to fourteen tissues for the control and Na^+-free conditions were calculated, respectively. Bars with the same letters (a and b) are not significantly different ($P < 0·05$).

than J^{Pr}_{ms}, resulting in a net secretory flux in Na^+-containing solution. In the distal colon J^{Pr}_{ms} was twice J^{Pr}_{sm}, and consequently a high net absorptive flux was seen. With the Na^+-free solution, on the other hand, J^{Pr}_{ms} and J^{Pr}_{sm} were similar and thus J^{Pr}_{ms} was not different from zero in the caecum and proximal colon. In the distal colon, however, both unidirectional fluxes of propionate decreased slightly, the considerable net absorptive flux remained unchanged.

In the caecum g_t increased from $7·8 \pm 0·3$ to $40 \pm 2·7$ mS cm^{-2} at incubation of the epithelium with Na^+-free solution. This drastic change in g_t may explain the increased J^{Pr}_{ms} in the caecum compared to the control values. In the proximal colon g_t changed from $14·2 \pm 0·7$ to $17·3 \pm 1·3$ mS cm^{-2}, and in the distal colon from $7·6 \pm 0·4$ to $11·8 \pm 0·9$ mS cm^{-2} under Na^+-free conditions. In all three segments I_{sc} was very small and merely different from zero.

Incubation of the epithelia with Na^+-free solutions thus caused similar changes in transport and electrical parameters to inhibition of the Na^+–K^+-ATPase with serosal ouabain.

DISCUSSION

The recently described segmental differences in unidirectional fluxes of SCFA across guinea-pig large intestinal epithelia (Engelhardt & Rechkemmer, 1992) could be confirmed in this study. (1) $J^{SCFA}_{ms} < J^{SCFA}_{sm}$ across the caecal and the proximal colonic epithelium, resulting in a net secretory flux from serosal to the mucosal side. (2) $J^{SCFA}_{ms} > J^{SCFA}_{sm}$ across the distal colonic epithelium, resulting in a considerable net absorptive flux. (3) J_{ms} in the distal colon increased with chain length ($J^{Bu}_{ms} > J^{Pr}_{ms} >$

J_{ms}^{Ac}), however, no such dependence was observed in the caecum and proximal colon. (4) In the proximal colon g_t was higher and I_{sc} was lower (Fig. 4) compared to the caecum and the distal colon.

Earlier *in vitro* (Rönnau *et al.* 1989) and *in situ* studies (Luciano *et al.* 1984) suggested that SCFA are absorbed across the distal colon of the guinea-pig primarily in the lipid soluble, non-ionized form, whereas in the proximal colon and in the caecum a considerable portion of SCFA may pass transcellularly also in the ionized form. In the present study the main emphasis was to elucidate the relationship between apical membrane H^+ ion secreting mechanisms and the mucosal to serosal flux of SCFA in the different segments of the guinea-pig large intestine. If non-ionic diffusion is a major mechanism of SCFA permeation, then a steady supply of H^+ ions would be necessary for a sustained SCFA absorption.

Na^+–H^+ exchange and SCFA transport

In the guinea-pig caecum and proximal colon Na^+ transport is electroneutral and most likely caused by the operation of a Na^+–H^+ exchange system located in the apical membrane of transporting enterocytes (Rechkemmer, 1988; Kuwahara & Radowicz-Cooke, 1988). In the present study it was confirmed that amiloride only caused a slight decrease in I_{sc} in the proximal colon. An interesting observation is the significantly reduced I_{sc} in the caecum at high concentration (1 mM) of amiloride. This may indicate the parallel presence of electrogenic Na^+ absorption and Na^+–H^+ exchange in the guinea-pig caecum. However, in contrast to the guinea-pig distal colon, in the caecum the electrogenic part of Na^+ absorption appears to be relatively insensitive to amiloride inhibition (Rechkemmer, 1988). Amiloride-insensitive electrogenic Na^+ transport has recently been demonstrated for the rabbit caecum (Sellin, Oyarzabal & Cragoe, 1988). In contrast to the rabbit caecum, however, in the guinea-pig caecum incubation with a Na^+-free solution led to a drastic increase in tissue conductance. Thus the guinea-pig caecum appears to be extremely dependent on Na^+ to maintain its normal physiological function. The nature of this large increase in g_t is unclear at present and remains to be established.

In any case, it is obvious that in the guinea-pig proximal colon and caecum, Na^+–H^+ exchange is a major mechanism responsible for transepithelial Na^+ transport, and thus the availability of H^+ ions for SCFA transport is determined by the operation of Na^+–H^+ exchange. In the present study in the guinea-pig caecum and proximal colon, amiloride significantly inhibited J_{ms} of all SCFA. In the rabbit proximal colon it also was recently demonstrated that propionate absorption was effectively inhibited by amiloride (Sellin & DeSoignie, 1990), indicating a significant contribution of H^+ secretion by Na^+–H^+ exchange to propionate absorption. Thus it appears that the activity of Na^+–H^+ exchange is an important factor in transepithelial transport of SCFA, at least in studies with intact rabbit or guinea-pig caecal or proximal colonic epithelium.

Species differences in respect to SCFA absorption may exist. In rats Na^+–H^+ exchange is not only present in the apical membrane of colonocytes of the proximal but, unlike guinea-pig, also of the distal segments (Binder, Stange, Murer, Stieger & Hauri, 1986; Rajendran & Binder, 1990). This may explain why in the rat distal colon SCFA stimulated active Na^+ and Cl^- absorption (Binder & Mehta, 1989, 1990). Amiloride inhibited the stimulation of Na^+ absorption by mucosal butyrate. That is

consistent with the supply of H^+ ions for non-ionic diffusion of SCFA (Binder & Mehta, 1989). In contrast to these findings recent experiments using apical membrane vesicles of rat distal colon indicate the presence of a bicarbonate-dependent SCFA anion transport system. This bicarbonate-dependent butyrate uptake into apical membrane vesicles was not inhibited by high amiloride concentrations, indicating that Na^+–H^+ exchange was not involved in butyrate transport across the apical membrane (Mascolo *et al.* 1991). Similarly, in human ileal brush-border membrane vesicles propionate transport was dependent on a bicarbonate gradient but was Na^+ independent (Harig, Soergel, Barry & Ramaswamy, 1991). On the other hand, in another acetate uptake study by brush-border membrane vesicles of rat small intestine results were consistent with acetate absorption occurring by non-mediated diffusion (Watson, Brennan, Farthing & Fairclough, 1991). This discrepancy between the vesicle studies cannot be readily explained and awaits further clarification.

Mechanisms involved in net secretion of SCFA in the caecum and proximal colon

In the present Ussing chamber experiments with symmetrical SCFA concentrations on both sides of the mucosa in the guinea-pig caecum and proximal colon J_{sm}^{SCFA} exceeds J_{ms}^{SCFA} resulting in a significant net secretory flux from the serosal to the mucosal site. This observation is in agreement with data obtained in the rabbit caecum (Hatch, 1987) and the rabbit proximal colon (Sellin & DeSoignie, 1990). Although the absolute rates of net acetate and propionate secretion were higher in the rabbit compared to the guinea-pig caecum, in both species an about twofold higher net secretory flux for acetate compared to propionate has been observed. The higher J_{sm} compared to J_{ms} for all three SCFA in the guinea-pig caecum and proximal colon appears to be related to a Na^+ gradient coupled transport system. Either inhibition of the basolateral Na^+–K^+-ATPase with ouabain or incubation in a Na^+-free solution eliminated net SCFA secretion. Under physiological conditions SCFA are present in high concentrations only at the luminal sides of the epithelium. Thus, the SCFA absorption from the large intestine reflects J_{ms}^{SCFA}. Due to the very low SCFA concentration in venous blood J_{sm}^{SCFA} under physiological conditions is very small, and no net secretion occurs in the caecum and proximal colon. However, the present studies under short-circuit current conditions allow us to draw conclusions on the mechanisms involved.

K^+–H^+-ATPase and SCFA transport

In the distal colon of the guinea-pig $J_{ms} > J_{sm}$ for all SCFA studied and thus a considerable net absorption was observed. Net SCFA absorption was not or only very slightly affected by mucosal amiloride treatment. Furthermore, incubation of the distal colon with Na^+-free solution also had no effect on unidirectional propionate fluxes, despite a complete inhibition of I_{sc}. The lack of an effect of amiloride and Na^+-free solution indicates that Na^+–H^+ exchange does not significantly contribute to SCFA transport in the distal colon of the guinea-pig. A potential source for H^+ ion secretion is the recently described ouabain-sensitive K^+–H^+-ATPase of the guinea-pig distal colon (Suzuki & Kaneko, 1987, 1989; Rechkemmer, 1988; Watanabe *et al.* 1990).

A concentration of 0·1 mM mucosal ouabain was used to minimize effects of ouabain permeating to the serosal side and affecting the basolateral Na^+–K^+ATPase.

Dose–response curves demonstrate that mucosal ouabain already at 0·1 mM concentration completely inhibited H^+ secretion (Suzuki & Kaneko, 1987, 1989), reduced ^{86}Rb uptake and inhibited ATPase activity to the same extent as 1 mM ouabain (Suzuki & Kaneko, 1989; Watanabe *et al.* 1990). At 0·1 mM mucosal ouabain, net Rb absorption across the distal colon of guinea-pig is completely inhibited (W. v. Engelhardt, M. Burmester, K. Hansen, G. Becker & G. Rechkemmer, unpublished results). Incubation of the guinea-pig distal colon epithelium in Na^+-free solution had no effect on net SCFA absorption (Fig. 4), indicating that the basolateral Na^+–K^+-ATPase activity is not related to SCFA mucosal to serosal fluxes; however, under these conditions 0·1 mM mucosal ouabain led to a similar inhibition of J_{ms}^{SCFA} as found under control conditions (W. v. Engelhardt, M. Burmester, K. Hansen, G. Becker & G. Rechkemmer, unpublished results). Therefore the employed concentration of ouabain is sufficient to elicit the effects demonstrated.

Addition of ouabain to the mucosal solution completely abolished net SCFA absorption, primarily due to a decrease in J_{ms}. This demonstrates the decisive importance of the K^+–H^+-ATPase for apical membrane uptake of SCFA. Mucosal ouabain had only minor effects in the caecum and no effect in the proximal colon, consistent with the absence of a K^+–H^+-ATPase in the proximal colon (Watanabe *et al.* 1990). A K^+–H^+ pump has also been described in the rat distal colon (Peronne & McBride, 1988; Foster, Dudeja & Brasitus, 1990). This K^+–H^+pump is Na^+ independent and inhibited by mucosal vanadate or ouabain (McLaughlin, McBride & Perrone, 1990). However, the relation of this system to SCFA transport has not yet been established in the rat.

Non-ionic diffusion or anion carrier-mediated transport of SCFA

In the present study the major emphasis was on the relation between apical membrane H^+ secreting systems and the mucosal to serosal SCFA fluxes. It is concluded from the data presented that Na^+–H^+ exchange in the caecum and proximal colon contributes by about 30–50% to J_{ms} of SCFA and thus non-ionic diffusion of the undissociated SCFA certainly constitutes an important factor in overall SCFA transport. However, J_{sm} is higher than J_{ms} under short-circuit current conditions in the caecum and proximal colon, and the net secretory flux is coupled (secondarily or even tertiary) to the Na^+ gradient established by the Na^+–K^+-ATPase, and most likely involves a basolaterally located anion exchanger.

In the distal colon net SCFA absorption is completely abolished by mucosal ouabain due to a marked inhibition of J_{ms}. In the experiments involving the presence of propionate on the mucosal side only, 80% of J_{ms} was inhibited by ouabain, suggesting that almost all propionate enters the enterocytes of the distal colon of guinea-pig by non-ionic diffusion. This conclusion is further supported by the marked increase of J_{ms} with the carbon chain length (Bu > Pr > Ac). That indicates that lipid solubility of the undissociated SCFA plays an important role in SCFA transport in the guinea-pig distal colon.

Experiments pursuing the issue of anion exchange systems and their involvement in SCFA transport across the different segments of the guinea-pig large intestine are currently being conducted.

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