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Absorption of short-chain fatty acids across ruminal epithelium of sheep

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Abstract Investigations on the absorption of short-chain fatty acids across ruminal epithelium of sheep were performed both in vitro (Ussing chamber technique, using propionic acid representatively for short-chain fatty acids) and in vivo (washed, isolated reticulorumen). A pH-induced, nearly tenfold increase in the concentration of undissociated propionate led to an only twofold increase in mucosal-to-serosal flux of propionate (in vitro). Neither amiloride (1 mmol·l^{-1}, in vitro) nor theophylline (10 mmol·l^{-1}, in vivo), inhibitors of the ruminal Na^+/H^+ exchanger, exerted any significant influence on propionate fluxes or short-chain fatty acids absorption, respectively. Total replacement of luminal Na^+ (by choline) did not alter short-chain fatty acids absorption (in vivo). Mucosal 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (0.1 mmol·l^{-1}) or mucosal nitrate (40 mmol·l^{-1}) markedly reduced propionate net flux (in vitro). Increasing mucosal Cl^- concentration brought about a significant drop in mucosal-to-serosal flux of propionate (in vitro) and in short-chain fatty acids net absorption (in vivo), respectively. The results obtained suggest that short-chain fatty acids are absorbed both as anions and as undissociated acids across ruminal epithelium of sheep. It is concluded that short-chain fatty acids anions either compete with Cl^- for binding sites at a common anion-exchange mechanism or that they are absorbed by an short-chain fatty acids anion/HCO_3^- exchanger indirectly coupled to a Cl^-/HCO_3^- exchanger via intracellular bicarbonate.

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Abbreviations *DIDS* 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid · *DMSO* dimethylsulfoxide · G_t tissue conductance · *HSCFA* protonated SCFA, i.e. undissociated form · J_{ms} mucosal-to-serosal flux · J_{sm} serosal-to-mucosal flux · J_{net} net flux · I_{sc} short-circuit current · *MOPS* (3-[*N*-morpholino]propanesulfonic acid) · *mu* mucosal · *Prop* Propionate · $SCFA^-$ SCFA anions, i.e. dissociated form · *SCFA* short-chain fatty acids · *SEM* standard error of mean

Introduction

In both large intestine and forestomach of ruminants, SCFA are produced by intraluminal microorganisms and are directly absorbed to a large extent (Bugaut 1987; Bergman 1990). As regards ruminants, up to 90% of the energy requirements can be met by SCFA absorbed from the rumen (Bergman 1990). Despite the great importance of SCFA for metabolism of the animal, mechanisms of ruminal SCFA absorption are not well understood, whereas SCFA transport in the large intestine has been characterized in detail (Bugaut 1987; Rajendran and Binder 1994; Rechkemmer 1994; Engelhardt 1995).

Regarding rumen, it is still a major question whether SCFA are absorbed in their protonated form (HSCFA) or as anions ($SCFA^-$). Assuming the former, protons are required to convert $SCFA^-$ into HSCFA because at physiological pH less than 1% of SCFA in the ruminal fluid is protonated. In various epithelia, the deficiency of protons is balanced by H^+-extruding mechanisms, particularly by a Na^+/H^+ exchanger located in the apical membrane. In proximal colon of pig and in proximal colon and caecum of guinea pig,

blocking of the Na^+/H^+ exchanger by amiloride reduced SCFA absorption (Holtug et al. 1992; Engelhardt et al. 1993). Although the existence of a Na^+/H^+ exchanger has been demonstrated in sheep rumen (Martens et al. 1991; Gäbel et al. 1991), it is still unclear whether this exchange mechanism plays a role in ruminal SCFA transport as demonstrated for the large intestine.

Besides the undissociated acid, SCFA can be absorbed in the dissociated form across gastrointestinal epithelia via electroneutral exchange mechanisms. The presence of anion-exchange mechanisms for $SCFA^-$ is suggested by investigations on human colon (Macfarlane and Cummings 1991) and ileum (Soergel et al. 1989; Harig et al. 1991) as well as on the intestine of the tilapia species *Oreochromis mossambicus* (Titus and Ahearn 1988). As for the rumen, Ash and Dobson (1963) as well as Aafjes (1967) assumed that luminal disappearance of SCFA and simultaneous appearance of bicarbonate are best explained by an exchange of SCFA anions for bicarbonate.

Therefore, the present study was designed to determine the form of SCFA (HSCFA or $SCFA^-$) absorbed from the rumen and the role of the Na^+/H^+ exchanger in this process. Furthermore, the contribution of anion-exchange mechanisms to the absorption of SCFA needed to be clarified. In the experiments carried out in vitro, propionate represented SCFA. In the in vivo studies, the sum of absorption of acetate, propionate, and butyrate absorbed was calculated and is further described as SCFA absorption.

Materials and methods

In vitro studies

Preparation of rumen epithelium

Sheep (*Ovis aries* aged 8–12 months, body weight 35–60 kg) were killed by ex sanguination after stunning and the reticulorumen was removed from the abdominal cavity 3–10 min later. A piece (150 cm^2) of the ventral rumen sac was cut out of the rumen wall and carefully washed in a buffer solution (37 °C; composition see below) gassed with 95% O_2/5% CO_2 (Messer-Griesheim, Germany). Epithelia were isolated and mounted in Ussing chambers as described by Gäbel et al. (1991).

Determination of propionate and Cl^- fluxes

After mounting epithelia were allowed to adapt to experimental conditions for 20 min. Pairs of epithelial sheets matching in conductances (difference less than 25%) were used for measurement of unidirectional fluxes: 18.4 kBq ^{14}C-propionate or 27.5 kBq ^{36}Cl were added to the mucosal or serosal side. Fluxes were calculated on the basis of radioactivity appearing on the unlabelled side according to Gäbel et al. (1991). Radioactivity was determined using a liquid scintillation counter (PW 4700, Philips, Netherlands) after adding Quickszint 2000 (Zinsser, Germany) to the samples.

Concerning propionate fluxes, it must be taken into consideration that part of propionate is metabolized in the ruminal epithelium (Stevens 1970). Therefore, radioactivity appearing at the unlabelled side represents both radioactive propionate and radioactive metabolites. Furthermore, propionate breakdown to CO_2 and subsequent removal of CO_2 by gassing lead to an underestimation of real fluxes. Therefore, the values calculated on the basis of radioactivity appearing on the "cold" side represent minimal values for the transport of both propionate and its metabolites.

Electrical measurements

Before mounting epithelia, junction potential and fluid resistance were determined for later automatic correction of electrophysiological measurements by the computer-controlled voltage-clamp device (AC-microclamp, f + p Datensysteme, Aachen, Germany). All flux measurements were carried out under short-circuit conditions. At regular intervals (150 s) epithelia were exposed to bipolar impulses of 100 μA for 1 s; the subsequently induced changes in transepithelial potential difference and the impulse amplitude served for calculating tissue conductance (G_t).

Buffer solutions

Buffer solution used for cleansing and transporting epithelia contained (mmol·l^{-1}): 75 NaCl, 25 $NaHCO_3$, 5 KCl, 2 NaH_2PO_4, 1 Na_2HPO_4, 1 $CaCl_2$, 2 $MgCl_2$, 10 glucose, 13 sodium butyrate, 13 sodium propionate, 13 sodium gluconate; pH 7.4, gassed with 95% O_2/5% CO_2.

Bathing solutions for Ussing-chambers were varied according to the experimental protocol and contained (mmol·l^{-1}):

First and second series (influence of mucosal pH and amiloride). 75 NaCl, 5 KCl, 2 NaH_2PO_4, 1 Na_2HPO_4, 1 $CaCl_2$, 2 $MgCl_2$, 10 glucose, 13 sodium butyrate, 13 sodium propionate, 13 sodium gluconate, 15 MOPS; serosal pH 7.5, gassed with 100% O_2. HCl was added to the mucosal side for adjusting pH to 7.5, 7.0 and 6.5. Equivalent amounts of choline chloride were added to the serosal side to prevent osmotic and chemical gradients.

Third series (influence of DIDS and nitrate on Cl and propionate transport). 25 $NaHCO_3$, 0.5 Na_2HPO_4, 1 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 112 sodium propionate, 5 potassium gluconate, 5 MOPS; pH 7.4, gassed with 95% O_2/5% CO_2. Propionate concentration was lowered by replacing sodium propionate with sodium gluconate.

In the experiments on nitrate, the following buffer solution served as control: 25 $NaHCO_3$, 0.5 Na_2HPO_4, 2 $CaCl_2$, 2 $MgCl_2$, 15 glucose, 1 sodium propionate, 110 sodium gluconate, 5 potassium gluconate, 8 MOPS; pH 7.4, gassed with 95% O_2/5% CO_2. To study the effect of nitrate, mucosal sodium gluconate was replaced by equimolar amounts of nitrate.

Fourth series (interactions between Cl^- and SCFA absorption). The interactions were tested by using the buffer solution of the third series in which mucosal Cl^- concentration was raised by replacing sodium gluconate by NaCl.

Inhibitors of ion-exchange mechanisms were dissolved in DMSO and then added to the mucosal buffer solution. Equal volumes of DMSO were added in the control group. The initial osmotic pressure of all solutions was determined by freezing-point depression (Knauer Osmometer, Germany) and adjusted to 290 ± 3 mosmol·l^{-1} by adding mannitol.

In vivo studies

Animals and feeding

Six Merino wether with permanent rumen fistulae in the dorsal rumen sac were used. The animals were fed concentrate (100 g oat per 50 kg body weight) twice a day. Hay and tap water were available ad libitum.

Determination of SCFA absorption

The washed, temporarily isolated reticulorumen technique was applied according to Care et al. (1984). After removing the fistula plug the reticulorumen was emptied and rinsed with buffer solution (39 °C). This procedure was repeated until buffer aspirated was free of visible rumen contents. The rumen contents removed were stored at 39 °C and refilled into the reticulorumen after finishing the experiment. In order to prevent flow of saliva into the reticulorumen, a saliva collector (Engelhardt and Sallmann 1972) was placed in the distal part of the oesophagus. Saliva accumulating cranially to the saliva collector was aspirated continuously and infused into the omasum via a balloon catheter fixed in the reticulo-omasal orifice. Moreover, this catheter prevented outflow of artificial rumen fluid during the experiment.

For the determination of net absorption, 2.5 l buffer solution (39 °C) were filled into the washed and isolated reticulorumen. Samples of the buffer solutions were taken shortly before putting the buffer into the reticulorumen and 3 and 63 min thereafter. They were stored at − 18 °C until analysis. Buffer solution in the reticulorumen was circulated continuously by gassing with 100% CO_2 (400 ml · min^{-1}, Air Liquide, Germany) via a silicone tube fitted with a diffusing nozzle.

Buffer solutions

Buffer solutions used were composed as follows (mmol · l^{-1}):
1. *Solution for cleansing the reticulorumen.* 100 NaCl, 20 $NaHCO_3$, 10 propionic acid.
2. *Experimental solution.* 25 $NaHCO_3$, 20 NaCl, 55 sodium acetate, 15 propionic acid, 5 butyric acid, 15 KOH, 5 K_2HPO_4, 2 $MgCl_2$, 2 $CaCl_2$, 1 chromium-EDTA. This solution served as basic buffer which was varied to establish specific experimental conditions by addition of theophylline (10 mmol · l^{-1}), by replacement of Na^+ (by choline), of Cl^- (by gluconate), or of SCFA (by gluconate) on a molar base. The initial osmotic pressure was adjusted to 290 ± 3 mosmol · l^{-1} by adding mannitol. Before infusion into the reticulorumen, buffer solutions were warmed to 39 °C and gassed with 100% CO_2 for 15 min.

Analyses and calculations

Na^+ and Cl^- concentrations were analyzed using ion-selective electrodes (NOVA 12, NOVA Biomedical, USA), chromium by atomic absorption spectrophotometry (AAS 30, Carl Zeiss Jena, Germany). SCFA concentrations were measured by capillary gas chromatography (Shimadzu GC15, Shimadzu Corp., Japan; capillary column: length 15 m; diameter 0.248 mm; liquid phase: DB-FFAP, i.e. polyethyleneglycol modified with nitroterephthalic acid; flame ionisation detector); isobutyric acid was used as internal standard. The osmotic pressure was determined by freezing-point depression (Knauer Osmometer, Germany).

For the calculation of net absorption, the actual volume of the buffer solution at the time of sampling was calculated based on the standard equation described by Care et al. (1984), using the analysed chromium concentrations.

Statistical analyses

As far as in vitro results are concerned, the *t*-test was applied for testing the differences between means of samples. The Newman-Keuls-test served for testing results shown in Table 1. The Wilcoxon-test was used for testing values obtained in vivo. All results are expressed as means with SEM; *N* represents the number of animals, *n* the number of observations.

Results

In vitro studies

First series: influence of mucosal pH on propionate fluxes

In order to increase the concentration of undissociated acid in the mucosal buffer solution, the pH of the mucosal buffer solution was gradually reduced from 7.5 to 6.5. Serosal pH was maintained at 7.5.

The gradual reduction of pH brought about a significant increase in J_{ms}^{Prop}, whereas J_{sm}^{Prop} was not significantly influenced (Table 1). If it is assumed that propionate is exclusively absorbed in the undissociated form, the increase in J_{ms}^{Prop} by lowering pH ought to be predictable using the Henderson–Hasselbalch equation. As shown in Table 1, the pH-induced increase in J_{ms}^{Prop} did not correspond to the increase in concentration of undissociated propionate as calculated using the Henderson–Hasselbalch equation. The calculated concentration increased nearly tenfold, whereas J_{ms}^{Prop} increased less than twofold.

Second series: influence of amiloride on propionate transport

In order to examine the influence of the Na^+/H^+ exchanger on ruminal SCFA absorption, the effect of amiloride on propionate transport was investigated in vitro. As shown in Table 2, amiloride (1 mmol · l^{-1}) added to the mucosal side did not exert any significant influence on unidirectional or net fluxes of propionate.

Third series: influence of DIDS and nitrate on Cl^- and propionate fluxes

Results of the first series of experiments suggested that propionate may also be absorbed in the anionic form. DIDS and nitrate are known inhibitors of anion-exchange mechanisms in several tissues (Aronson and Seifter 1984; Jennings 1992). As regards rumen

Table 1 Effect of mucosal pH on propionate (prop) fluxes, short-circuit current (I_{sc}), and conductance (G_t) in isolated sheep rumen epithelium in vitro. Serosal pH was kept at 7.5. Values represent means ± SEM; $N = 9/n = 18$

Mucosal pH	Mucosal concentration of undissociated propionate[d] ($mmol \cdot l^{-1}$)	J_{ms}^{Prop}	J_{sm}^{Prop} ($\mu mol \cdot cm^{-2} \cdot h^{-1}$)	J_{net}^{Prop}	I_{sc} ($\mu eq \cdot cm^{-2} \cdot h^{-1}$)	G_t ($mS \cdot cm^{-2}$)
7.5	0.026	0.85 ± 0.06[a]	1.56 ± 0.10	− 0.72 ± 0.11	0.99 ± 0.08	2.86 ± 0.19
7.0	0.082	1.05 ± 0.08[b]	1.66 ± 0.07	− 0.61 ± 0.11	0.84 ± 0.09	2.99 ± 0.19
6.5	0.254	1.29 ± 0.08[c]	1.72 ± 0.12	− 0.44 ± 0.12	0.88 ± 0.07	2.70 ± 0.15

[a, b, c] different letters indicate differences of at least $P < 0.05$
[d] calculated using the Henderson–Hasselbalch equation (total concentration of propionate: 13 $mmol \cdot l^{-1}$; pK 4.8)

Table 2 Effect of mucosal amiloride (1 $mmol \cdot l^{-1}$) on propionate fluxes and on electrophysiological parameters in isolated sheep rumen epithelium in vitro. Values represent means ± SEM; $N/n = 9$

	J_{ms}^{Prop}	J_{sm}^{Prop} ($\mu mol \cdot cm^{-2} \cdot h^{-1}$)	J_{net}^{Prop}	I_{sc} ($\mu eq \cdot cm^{-2} \cdot h^{-1}$)	G_t ($mS \cdot cm^{-2}$)
Control	1.13 ± 0.08	1.55 ± 0.08	− 0.42 ± 0.08	0.62 ± 0.10	3.04 ± 0.28
Amiloride	1.04 ± 0.07	1.43 ± 0.11	− 0.39 ± 0.13	0.45 ± 0.10	3.31 ± 0.39

Table 3 Effect of mucosal addition of DIDS (0.1 $mmol \cdot l^{-1}$) on Cl^- and propionate fluxes across isolated, short-circuited sheep rumen epithelium in vitro. Cl^- fluxes were measured both at high (112 $mmol \cdot l^{-1}$) and low (2 $mmol \cdot l^{-1}$) propionate concentrations. Values represent means ± SEM

Concentration ($mmol \cdot l^{-1}$)	Treatment	J_{ms}	J_{ms} ($\mu mol \cdot cm^{-2} \cdot h^{-1}$)	J_{net}	I_{sc} ($\mu eq \cdot cm^{-2} \cdot h^{-1}$)	G_t ($mS \cdot cm^{-2}$)	N/n
Chloride fluxes							
$[Cl^-]$ / [Prop]:	Control	0.66 ± 0.04[a]	0.42 ± 0.02[a]	0.24 ± 0.09	0.92 ± 0.20	2.12 ± 0.15	9/9
4/2	DIDS	0.42 ± 0.06[b]	0.24 ± 0.02[b]	0.19 ± 0.05	0.90 ± 0.10	2.07 ± 0.09	9/9
$[Cl^-]$ / [Prop]:	Control	0.23 ± 0.05	0.16 ± 0.03	0.08 ± 0.03	0.50 ± 0.11	2.39 ± 0.29	6/6
4/112	DIDS	0.22 ± 0.02	0.14 ± 0.03	0.08 ± 0.01	0.46 ± 0.09	2.13 ± 0.16	6/6
Propionate fluxes							
$[Cl^-]$ / [Prop]:	Control	0.217 ± 0.018[a]	0.134 ± 0.007[a]	0.073 ± 0.01[a]	0.97 ± 0.19	1.69 ± 0.14	9/9
4/2	DIDS	0.091 ± 0.021[b]	0.066 ± 0.009[b]	0.024 ± 0.02[b]	0.98 ± 0.18	1.72 ± 0.20	9/9

[a, b] different letters indicate differences at least $P < 0.01$

epithelium, the effect of DIDS on ruminal Cl^- transport could not definitely be proven (Martens et al. 1991).

Mucosal addition of DIDS induced a significant drop in J_{ms}^{Cl} as well as in J_{sm}^{Cl} (Table 3), but these effects of DIDS were observed only at a very low concentration of both chloride (4 $mmol \cdot l^{-1}$) and propionate (2 $mmol \cdot l^{-1}$) (Table 3).

The effects of DIDS on propionate transport were determined under conditions under which the stilbene derivative inhibits Cl^- transport, i.e. at low concentrations of both propionate and chloride (2 $mmol \cdot l^{-1}$ and 4 $mmol \cdot l^{-1}$, respectively). Under these conditions, DIDS significantly decreased both unidirectional and net fluxes of propionate (Table 3).

Substitution of nitrate (40 $mmol \cdot l^{-1}$) for gluconate in the mucosal bathing solution led to effects similar to those observed with DIDS, i.e. reduction of both unidirectional propionate fluxes. J_{ms}^{Prop} was more markedly reduced than J_{sm}^{Prop} leading to a net secretion of propionate (Fig. 1).

Fourth series: interactions between Cl^- and SCFA absorption

Elevation of mucosal Cl^- concentration from 4 to 44 $mmol \cdot l^{-1}$ resulted in a significant reduction of J_{ms}^{Prop} and J_{net}^{Prop} (Fig. 2A); J_{sm}^{Prop} was not influenced. Moreover, a remarkable drop in J_{ms}^{Cl} was induced by increas-

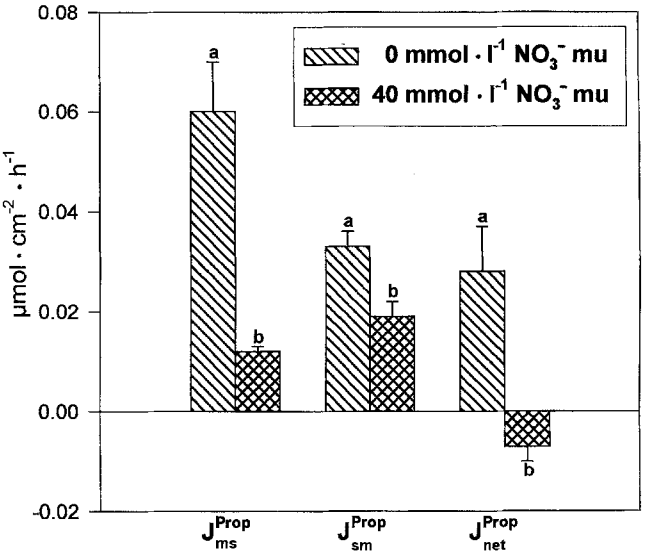


Fig. 1 Effect of mucosal (*mu*) nitrate on unidirectional and net propionate fluxes across isolated, short-circuited sheep rumen epithelium in vitro (*bars* represent means + SEM; $N/n = 7$; *a*, *b*: different values indicate significant differences of $P < 0.01$; propionate (*prop*) concentration: 1 mmol·l^{-1}, Cl^- concentration: 4 mmol·l^{-1})

ing mucosal propionate concentration from 2 to 112 mmol·l^{-1}, where J_{sm}^{Cl} decreased only slightly (Fig. 2B). The drop in J_{ms}^{Cl} led to a complete suppression of J_{net}^{Cl}.

In vivo studies

First series: effect of theophylline on SCFA transport

Since amiloride may cause toxic effects, theophylline was used as a blocking agent of the Na^+/H^+ exchanger in the in vivo studies. As known from previous in vitro investigations on sheep rumen epithelium (Gäbel et al. 1992), theophylline leads to an inhibition of the amiloride-sensitive Na^+ transport. In the washed, temporarily isolated reticulorumen, addition of 10 mmol·l^{-1} theophylline to the buffer solution caused a significant decrease in net Na^+ absorption, whereas SCFA absorption remained almost constant (Fig. 3).

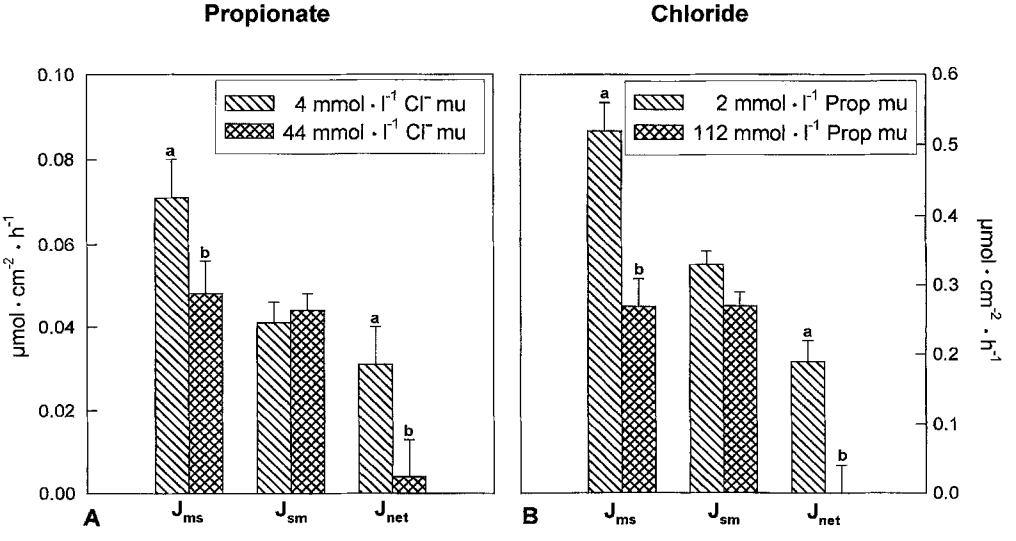
Second series: effect of Na^+ and Cl^- on SCFA transport

Theophylline also exerts inhibitory effects on electrogenic transport mechanisms (Martens et al. 1989; Wolffram et al. 1989). To exclude this effect, Na^+ was totally substituted by choline in an additional experiment. Despite the total absence of Na^+, there was no change in SCFA absorption (Table 4). In contrast to Na^+, Cl^- showed a marked effect on SCFA transport. SCFA net absorption was greater in the absence of Cl^- (Table 4).

Discussion

After mucosal acidification, absorption of propionate did not increase to the extent expected from the

Fig. 2A, B Cl^- and propionate fluxes across isolated, short-circuited sheep rumen epithelium at various mucosal concentration of Cl^- or propionate (*bars* represent mean + SEM; *a*, *b*: different values indicate significant differences of $P < 0.01$): **A** propionate fluxes at low or high mucosal Cl^- concentration; serosal Cl^- concentration was maintained constant at 4 mmol·l^{-1}. Propionate concentration on both sides of the epithelium was 2 mmol·l^{-1} ($N = 6, n = 12$); **B** Cl^- fluxes at low or high mucosal propionate concentrations; serosal propionate concentration was maintained constant at 2 mmol·l^{-1}. Cl^- concentration on both sides of the epithelium was 4 mmol·l^{-1} ($N = 6, n = 12$)



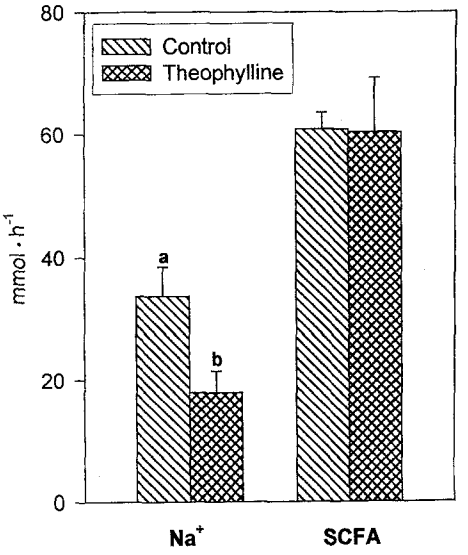


Fig. 3 Effect of theophylline (10 mmol·l^{-1}) on net absorption of Na^+ and SCFA from the washed, temporarily isolated reticulorumen of sheep in vivo (*bars* represent mean + SEM; *a*, *b*: different values indicate significant differences of $P < 0.05$; $N/n = 6$)

Table 4 Effect of Na^+ and/or Cl^- replacement on SCFA net absorption in vivo. Values represent means ± SEM; $N/n = 6$

Na^+ concentration (mmol·l^{-1})	Cl^- concentration (mmol·l^{-1})	SCFA net absorption (mmol·h^{-1})
100	28	68.9 ± 10.3[a]
0	28	87.6 ± 9.2[a, b]
100	0	94.9 ± 5.2[b]

[a, b] different superscripts indicate differences of $P < 0.05$

calculated increase in the concentration of the undissociated form. Similar observations have been reported by Stevens and Stettler (1966) on isolated cattle rumen epithelium, as well as by Weigand et al. (1972) and by Thorlacius and Lodge (1973) in rumen of cows under in vivo conditions. All these results suggest that the absorption of SCFA does not entirely depend on the transepithelial concentration gradient of SCFA. However, transepithelial gradient is not the only driving force for SCFA transport. Since transepithelial permeation of SCFA is probably located on a transcellular route, they must first to be taken up across the apical membrane(s) into the cytosol. Because of their lipid solubility, HSCFA concentration in the mucosal solution and the cytosol may equalize rapidly. Consequently, their concentration gradient across the apical membrane(s) diminishes, leading to a minor driving force for HSCFA.

pH-independent transport of HSCFA might also be due to a mechanism similar to that postulated in studies on proximal and distal colon of guinea pig (Rechkemmer and Engelhardt 1988; Engelhardt et al. 1993). In these tissues, SCFA absorption is virtually independent of luminal pH due to the presence of proton-extruding mechanisms in the apical membrane, contributing to protonation of $SCFA^-$ near the epithelial surface. Although the existence of a Na^+/H^+ exchanger sensitive to amiloride and theophylline has been proven in previous studies on rumen epithelium (Martens et al. 1991; Gäbel et al. 1991, 1992), neither amiloride, theophylline nor complete replacement of Na^+ exerted any influence on propionate or SCFA transport in the present study (Tables 2, 4; Fig. 3). Consequently, transport of undissociated acids linked to the activity of the Na^+/H^+ exchanger, as described for the large intestine, is not present in the rumen at least under the conditions chosen.

In addition to being transported in the protonated form, SCFA may also traverse rumen epithelium in anionic form with the help of anion-exchange mechanisms similar to those known from investigations on other gastrointestinal epithelia (Titus and Ahearn 1988; Soergel et al. 1989; Macfarlane and Cummings 1991; Harig et al. 1991). Such anion-exchange mechanisms can be successfully blocked by stilbene derivatives such as DIDS (Jennings 1992). However, Martens et al. (1991) failed to demonstrate a blocking effect of DIDS on Cl^- net transport in rumen epithelium. Therefore, we repeated these experiments but with reduced Cl^- and SCFA concentrations. Under these conditions, DIDS significantly reduced all Cl^- fluxes determined (Table 3), indicating the existence of DIDS-sensitive anion-exchange systems.

DIDS did not only significantly reduce chloride fluxes but also induced a drop in propionate transport (Table 3). Similar effects were induced by nitrate in the mucosal buffer solution (Fig. 1) which has also been shown to block anion exchange systems (Aronson and Seifter 1984). From these results, it can be concluded that propionate anions can be absorbed by anion-exchange mechanisms.

Since DIDS and nitrate influenced both propionate and Cl^- transport in a similar way (Würmli et al. 1987; present study), an interrelationship between the transport of these substances can be assumed. Interactions are further suggested by the observation that mucosal (luminal) Cl^- decreased SCFA/propionate transport and, vice versa, elevation of propionate concentration decreased Cl^- transport (Fig. 2; Table 4).

The interrelationship between Cl^- and SCFA/propionate transport can be explained in at least two ways: firstly, a direct interaction of Cl^- and $SCFA^-$ at a common anion exchanger. If the exchanger were able to transport not only Cl^- but also $SCFA^-$, then both ions would compete for the binding sites and, depending on the respective concentration, displace each other. Secondly, Cl^- and $SCFA^-$ may interact by an indirect coupling of two different mechanisms. Besides the Cl^-/HCO_3^- exchanger (Chien and Stevens 1972; Würmli et al. 1987), a $SCFA^-/HCO_3^-$ exchanger might

exist in sheep ruminal epithelium similar to those in the small and large intestine as described by Harig et al. (1991) and Mascolo et al. (1991). If two transporters exist, interactions between Cl^- and SCFA transport might be explained by the need for HCO_3^- by both exchangers inside the ruminal epithelial cell. Assuming the intracellular HCO_3^- production from CO_2 is very small, elevation of luminal Cl^- concentration may lead to a decrease in intracellular HCO_3^- concentration due to an elevated transport rate of the Cl^-/HCO_3^- exchanger. Consequently, less intracellular HCO_3^- would be available for the $SCFA^-/HCO_3^-$ exchanger resulting in a reduction of SCFA transport. Vice versa, an increase in luminal $SCFA^-$ concentration would bring about a similar effect on Cl^- transport. At present, we cannot verify whether $SCFA^-$ are absorbed by a non-specific Cl^-, $SCFA^-/HCO_3^-$ exchanger and/or by a specific $SCFA^-/HCO_3^-$ exchanger.

In conclusion, our results demonstrate that SCFA are absorbed across ruminal epithelium in both protonated and anionic forms. The quantity of absorbed SCFA depends partially on luminal pH, the remaining part being probably transported via anion-exchange mechanism(s). The influence of DIDS and nitrate on propionate fluxes demonstrates that a considerable amount of propionate is absorbed by such a mechanism under the conditions chosen. However, it was necessary to lower both propionate and Cl^- concentration to achieve an effect of DIDS and nitrate. This suggests that anion-exchange systems play a minor role under physiological conditions, since significantly higher concentrations of SCFA are normally found in the lumen of the reticulorumen. On the other hand, Cl^- exerted a distinct effect on propionate absorption in vivo at physiological concentrations of SCFA. This points to a Cl^--sensitive absorption of SCFA in its anionic form also under in vivo conditions. In contrast to various studies on the large intestine, SCFA transport is independent of the presence of sodium and of the activity of a Na^+/H^+ exchanger. Despite the independence of SCFA from Na^+, interactions between Na^+ and SCFA occur in the opposite direction, i.e. SCFA strongly stimulate Na^+ transport (Gäbel et al. 1991; Sehested et al. 1996).

Besides the mechanisms of SCFA transport discussed above, intraepithelial metabolism of SCFA seems to affect their absorption (Stevens and Stettler 1966; Bergman 1990). To minimize metabolic effects we chose propionate for the in vitro studies since it is metabolized to only a small extent (Bergman 1990). Nevertheless, our conclusions are based on the prerequisite that the manipulations (ion replacement or addition of inhibitors) do not influence the ratio of unmetabolized to metabolized SCFA transported by the epithelium.

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