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Influence of isoform and DNP on butyrate transport across the sheep ruminal epithelium

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Abstract Short-chain fatty acids are absorbed in considerable amounts from the rumen. During transit through the epithelial layer, they are intensively metabolised. Interaction between intraepithelial metabolism and absorption, however, is hardly understood. The present study therefore compared the transepithelial transport of the easily metabolised $n$-butyrate with that of the more metabolism-resistant iso-butyrate both under in vivo conditions (isolated and washed reticulorumen) and in vitro conditions (Ussing chamber). Under in vivo conditions, net absorption of $n$-butyrate was significantly higher than that of iso-butyrate. The in vitro experiments showed that the higher net flux of $n$-butyrate was solely due to a higher mucosal-to-serosal flux, whereas the serosal-to-mucosal flux of butyrate was independent from the isoform. Blocking intraepithelial ATP delivery by 2,4-dinitrophenol abolished the net flux of $n$-butyrate. The study indicates that metabolism and/or ATP availability stimulates $n$-butyrate net absorption. By this, the metabolic activity of the epithelium may have a regulatory influence on absorption of $n$-butyrate.

Key words SCFA · Ketone bodies · $n$-butyrate · Monocarboxylate transport · Anion exchange

Abbreviations Ac acetate · DIDS 4,4′-diisothiocyantostilbene-2,2′-disulfonic acid · DNP 2,4-dinitrophenol · $G_i$ tissue conductance · iso-Bu iso-butyrate · $J$ flux · $MCT H^+$ monocarboxylate transporter · MOPS (3-[N-morpholinolpropanesulfonic acid) · mS mucosal-to-serosal · n-Bu $n$-butyrate · $Pr$ propionate · SCFA− SCFA anions, i.e., dissociated form · SCFA short-chain fatty acids · mS serosal-to-mucosal

Introduction

In the ruminant forestomach, ingested carbohydrates are almost completely broken down to the three major short-chain fatty acids (SCFA), acetate, propionate and $n$-butyrate and the gases, carbon dioxide, methane and molecular hydrogen (Fahey and Berger 1988). A great part of intraruminally produced SCFA is directly absorbed across the reticuloruminal wall and largely covers the energy demand of the animal (Bergman 1990).

During transit across the forestomach wall, all three SCFA undergo an intensive intraepithelial metabolism (Bergman 1990). Among the SCFA, however, $n$-butyrate is metabolised most intensively. On the basis of various in vitro and in vivo findings, it can be calculated that up to 95% of $n$-butyrate taken up into the cell is metabolised by the ruminal epithelium to ketone bodies (acetoacetate and 3-hydroxybutyrate) and carbon dioxide (Stevens 1970; Weekes 1974; Weigand et al. 1975; Beck et al. 1984; Bergman 1990; Baldwin and Jesse 1992; Kristensen et al. 1996; Sehested et al. 1999b).

So far, not much is known about how the intraperithelial metabolism modulates the transepithelial transport of $n$-butyrate. Due to the modulating effect of metabolism, the transepithelial transport of $n$-butyrate does not simply result from the action of transport proteins and permeabilities as in the case of electrolyte transport. Consequently, the models for transruminal transport of $n$-butyrate are controversial and still incomplete (Gäbel and Sehested 1997; Sehested et al. 1999a, 1999b).

To get insight into the interaction between metabolism and transport of butyrate, the present study compared the transepithelial transport of $n$-butyrate and iso-butyrate under both in vivo and in vitro conditions. Iso-butyrate is metabolised to a much lesser extent than $n$-butyrate in gastrointestinal epithelia including the

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ruminal epithelium (Weigand et al. 1975; Chu and Montrose 1995; Charney et al. 1998; Sehested et al. 1999b). Experimental comparison of n-butyrates and iso-
butyrates transport should, therefore, help to clarify the role of metabolism in butyrates transport. Additionally, the effect of 2,4-dinitrophenol (DNP) was tested to de-
termine whether metabolism/transport interaction is via the produced metabolites per se or whether it is via the supply of metabolic energy. DNP has been shown to uncouple oxidative phosphorylation and so to inhibit ATP delivery (Abo-Khatwa et al. 1996).

Materials and methods

In vivo studies

Animals and pre-experimental feeding

Six Merino wethers (Ovis aries) with permanent ruminal fistulae in the dorsal ruminal sac were used. The animals were fed concentrate (100 g oat/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 the procedure described by Care et al. (1984). After removing the fistula plug, the reticulorumen was emptied. The reticulorumen contents were stored at 39 °C and replaced in the reticulorumen after the completion of the experiment. The emptied reticulorumen was rinsed with cleansing buffer solution (39 °C; composition: see “Buffer solutions” section). The cleansing procedure was repeated until the buffer aspirated was free of visible ruminal contents. In order to prevent the flow of saliva into the reticulorumen, a saliva collector (Engelhardt and Saltmann 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 the outflow of artificial ruminal fluid during the experiment.

For the determination of net absorption, 2.5 l experimental buffer solution (39 °C; composition: see “Buffer solutions” section) was filled into the washed and isolated reticulorumens. Samples of the buffer solutions were taken shortly before putting the buffer into the reticulorumens and 3 min and 63 min thereafter. They were stored at −18 °C until analysis. Buffer solution in the reticulorumen was circulated continuously by gassing with 100% CO₂ (400 ml-

Buffer solutions

Buffer solutions used were composed as follows (mM:1⁻): solution for cleaning the reticulorumen: 100 NaCl, 20 NaHCO₃, 10 propionic acid; standard experimental solution: 25 NaHCO₃, 20 NaCl, 50 sodium gluconate, 5 K₂HPO₄, 2 MgCl₂, 2 CaCl₂, 1 chromium-EDTA, 15 sodium n-butyrate and/or sodium iso-butyrate. When the experimental solution contained both 15 mM sodium n-butyrate and 15 mM sodium iso-butyrate, the concentration of sodium gluconate was lowered to 35 mM⁻¹.

The initial osmotic pressure of all solutions was adjusted to 260 ± 3 mosmol⁻¹ by adding mannitol. Before infusion into the reticulorumen, buffer solutions were warmed to 39 °C and gassed with 100% CO₂ for 15 min. Initial pH was adjusted to 6.70 ± 0.03. During the 60-min stay in the reticulorumen, pH increased by 0.01−

Analyses and calculations

Chromium concentration was analysed by atomic absorption spectrophotometry (AAS 30, Carl Zeiss Jena, Germany). Concentration by ion selective electrodes (NOVA 12, NOVA biomediki, USA). Concentrations of n-butyric acid and iso-butyric acid were measured by capillary gas chromatography (Shimadzu GC15 with flame ionisation detector, Shimadzu Japan; capillary column: length 15 m; diameter 0.248 mm; liquid phase: DB-FFAP, i.e., polyethylene glycol modified with nitroterephthalic acid). 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 calculated chromium concentrations using the standard equation described by Care et al. (1984).

In vitro studies

Preparation of the ruminal epithelium

Sheep (O. aries), aged 8–12 months with body weights of 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 cm²) of the ventral ruminal sac was cut out of the ruminal wall and carefully washed in an SCFA-containing buffer solution (37 °C; for composition see below) gassed with 95% O₂/

Determination of 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. 46 kBu of the respective ¹⁴C-labelled SCFA was added to the mucosal or serosal side. Fluxes were calculated on the basis of radioactivity appearing at the unlabelled side according to Gäbel et al. (1991). Radioactivity was measured by liquid scintilllation counting (Wallac 1409 LSC, Berthold, Bad Wilsch, Germany) after adding liquid scintillation fluid (Aquasafe 300 Plus, Zinsser Analytic, Maidenhed, UK). Concerning SCFA fluxes, it must be taken into consideration that radioactivity appearing at the unlabelled side represents both the radioactive SCFA and those radioactive metabolites that are not able to escape the system, i.e., non-CO₂ metabolites. Therefore, in using the term “SCFA flux”, we refer to the flux of SCFA plus the flux of their non-CO₂ metabolites.

Electrical measurements

Before mounting the epithelia, junction potential and fluid resis-
tance were determined for automatic correction of electrophysi-
ological measurements by the computer-controlled voltage-clamp device (AC-microclamp, f + p Datensysteme Aachen, Germany). All flux measurements were carried out under short-circuit condi-
tions. At regular intervals (150 s) epithelia were exposed to bipolar impulses of 100 μA for 1 s. The induced changes in transepithelial potential difference and the impulse amplitude served for the calculation of tissue conductance (GΩ).

Buffer solutions

Standard buffer solution used for determination of n-butyrate or iso-butyrate fluxes contained (mM:1⁻): 75 NaCl, 25 NaHCO₃, 5 KCl, 2 NaH₂PO₄, 1 Na₂HPO₄, 1 CaCl₂, 2 MgCl₂, 53-[N-mor-
pholino]propanesulfonic acid (MOPS), 30 sodium gluconate, 10 sodium n-butyrate or sodium iso-butyrate; gassed with 95% O₂/5% CO₂. The effect of 4,4'-disothiocyanatostilbene-2,2'-disulphonic acid (DIDS) shown in Fig. 4 was determined in a similarly composed