Research in Veterinary Science 117 (2018) 10–17

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Permeation of acetate across sheep ruminal epithelium is partly mediated by an anion channel

Reiko Rackwitz*, Gotthold Gäbel

University of Leipzig, Institute of Veterinary Physiology, Germany

ARTICLE INFO

Keywords:
Rumen
Anion channel
Short-chain fatty acids (SCFA)
Membrane voltage

ABSTRACT

The present study pursued the question if an anion channel could be partly responsible for the transport of acetate in the isolated ovine ruminal epithelium. Using the Ussing chamber technique, changes of short-circuit current (I_{sc}) induced by mucosal or serosal addition of acetate was investigated. To further evaluate the I_{sc} changes induced by acetate the epithelia were preincubated with ouabain or 4,4′-diisothiocyano-2,2′-stilbene-disulfonic acid (DIDS). In addition, unidirectional flux rates of ^{14}C-acetate were measured at different transepithelial potential difference (V_t). At V_t = 0 mV, acetate addition to the mucosal side of epithelia incubated in ouabain/DIDS free solution resulted in an I_{sc} decrease, whereas application to the serosal side induced an I_{sc} increase. Absolute I_{sc} changes were significantly larger after serosal than after mucosal acetate addition. Ouabain pre-incubation abolished these side-specific differences. Pre-incubation with DIDS on the mucosal side inhibited the current induced by subsequent mucosal acetate addition in a voltage dependent manner, whereas serosal DIDS pre-incubation had no effect on acetate-induced changes of I_{sc}. Mucosal-to-serosal acetate flux was partly voltage-dependent. The serosal-to-mucosal flux of acetate was not influenced by V_t. The asymmetric changes of I_{sc} after acetate application and the V_t dependence of the DIDS inhibition on the mucosal side indicate a partly electrogenic transcellular permeation of acetate which includes an apically located acetate conductance. It is suggested that this conductance may be important for epithelial cell homeostasis.

1. Introduction

When ruminats are fed on physiological diets, up to 80% of ingested carbohydrates are anaerobically degraded in the reticulorumen by various microorganisms while only a small part remains for enzymatic digestion in the small intestine (Harmon et al., 2004). The main products of the fermentational process in the reticulorumen are short-chain fatty acids (SCFA), primarily acetate, propionate and butyrate. Most of the SCFA produced are directly absorbed from the rumen and cover the major part of ruminant's energy requirements. Depending on the diet, the total concentration of the three SCFA ranges from ~ 70 to 120 mmol l^{-1} in the rumen of sheep (Bergman, 1990; Chibisa et al., 2016). With a proportion of ~70%, acetate is the most abundant SCFA in the ruminal fluid (Penner et al., 2009b). Up to 90% of ruminally produced acetate can be absorbed directly from the rumen (Penner et al., 2011).

In the ruminal fluid, SCFA are present both in the protonated form (HSCFA) and in the electrically charged form ($SCFA^-$). Since HSCFA are rather lipophilic and may permeate biological membranes directly (Walter and Gutknecht, 1986), the mechanism of simple diffusion was discussed as a main uptake pathway for SCFA for a long time (e.g. Stevens and Stettler, 1966a; Ali et al., 2006; Graham et al., 2007). However, at the physiological ruminal pH of 6.1–6.8 (Penner et al., 2009a; Steele et al., 2012), the major part of SCFA is available as SCFA anions due to their pK_a of ~4.8 (Aschenbach et al., 2011).

The two forms of SCFA represent an equilibrium that will be adjusted again whenever simple diffusion of HSCFA occurs. However, a partial transport of $SCFA^-$ across the ruminal epithelium via anion-transporting proteins can be assumed (Aschenbach et al., 2009; Aschenbach et al., 2011; Dengler et al., 2013). Significant permeation of $SCFA^-$ explains that only small increases in SCFA absorption can be achieved when increasing chain length (Sehested et al., 1999a) or decreasing pH (Stevens and Stettler, 1966a; Kramer et al., 1996; Sehested et al., 1999a), i.e. that permeation does not fit the lipophilicity differences and Hendersson-Hasselbalch equilibria of SCFA assuming exclusively passive diffusion.

As reviewed by Aschenbach et al. (2011), several protein-mediated transport mechanisms contribute significantly to total SCFA absorption from the rumen. Some studies suggested a bicarbonate-dependent uptake of $SCFA^-$ (Gäbel et al., 1991; Kramer et al., 1996; Sehested et al.,

* Corresponding author at: Veterinär-Physiologisches Institut, Universität Leipzig, An den Tierkliniken 7, 04103 Leipzig, Germany.
E-mail address: rackwitz@vetmed.uni-leipzig.de (R. Rackwitz).

https://doi.org/10.1016/j.rvsc.2017.11.004
Received 25 May 2016; Received in revised form 24 October 2017; Accepted 10 November 2017

1999b; Aschenbach et al., 2009) mediated by a direct HCO_3^-/$SCFA^-$ exchange (Bilk et al., 2005; Aschenbach et al., 2009). The study of Aschenbach et al. (2009) provided evidence for an additional bicarbonate-independent, nitrate-inhibitable component of SCFA absorption. According to calculations by Penner et al. (2009a), this pathway may account for up to 19% of total acetate uptake in the ovine rumen. However, the nature and mechanism of this way of permeation have not been elucidated so far. One possible candidate for that pathway permeable to $SCFA^-$ is an anion channel. $SCFA^-$ have been shown to permeate anion channels in Calu-3 cells (Illek et al., 1999) and mouse cholangiocytes (Chen et al., 2004). Also, in cultured ruminal epithelial cells, the expression of an $SCFA^-$ permeable anion channel was functionally proved (Stumpff et al., 2009; Georgi et al., 2013).

Therefore, the present study pursued the question if the intact ovine ruminal epithelium may be able to transport $SCFA^-$ via electrogenic pathways. Due to its high abundance in the ruminal fluid and due to its low lipophilicity in comparison to other SCFA, we used acetate to explore this issue.

2. Material and methods

2.1. Ethical approval

Experiments were according with the German legislation on the protection of animals and the EU Directive 2010/63/EU for animal experiments. The studies were communicated to the Regierungspräsidium Leipzig under file number AZ 24-9162.11-01-T 58/04 and T86/10.

2.2. Chemicals and buffer solutions

Unless otherwise noted, all chemicals were supplied by Merck (Darmstadt, Germany), Carl Roth (Karlsruhe, Germany), or Sigma-Aldrich (Schnelldorf, Germany). ^{14}C-acetate was obtained from Hartmann Analytic (Braunschweig, Germany).

Buffer solution for washing and transport of the epithelia ("transport buffer solution") contained (mmol l^{-1}): 80 sodium gluconate, 5.5 potassium gluconate, 50 NaCl, 1.25 calcium gluconate, 1.25 magnesium gluconate, 0.6 NaH_2PO_4, 2.4 Na_2HPO_4, 1 glutamine, 10 HEPES, 5 choline base, 10 glucose. Incubation buffer solution was nominally chloride- and bicarbonate-free and contained (mmol l^{-1}): 10 sodium gluconate, 5.5 potassium gluconate, 1.25 calcium gluconate, 1.25 magnesium gluconate, 0.6 NaH_2PO_4, 2.4 Na_2HPO_4, 1 glutamine, 10 HEPES, 122 choline base, 10 glucose, 111 gluconic acid. To avoid epithelial bicarbonate production by carbonic anhydrase, ethoxyzolamide (0.06 mmol l^{-1}) was added. For acetate addition, an acetate-rich buffer solution was created in the same way by substituting sodium acetate, potassium acetate and acetic acid for sodium gluconate, potassium gluconate and gluconic acid, respectively. Likewise, a potassium-rich buffer solution (100 mmol l^{-1} potassium) was composed by substitution of choline base for potassium and used to depolarize the apical or basolateral membranes in the respective experiments. All buffer solutions had a pH of 7.4 and were gassed with 100% oxygen.

The Na^+-K^+ ATPase blocker ouabain was diluted in water before use and exclusively added to serosal side with a final concentration of 0.1 mmol l^{-1}. Disodium 4,4′-diisothiocyanatostilbene-2,2′-disulfonate (DIDS) was added directly to the buffer solution to achieve a final concentration of 1 mmol l^{-1}.

2.3. Animals and treatment of epithelia

Epithelia from adult female Merino breed sheep (*Ovis aries*) were used for the experiments. Sheep were fed with hay and water ad libitum for at least three weeks prior to the experiments. They were killed by captive bolt stunning followed by exsanguination. Ruminal tissue from the ventral sac was removed immediately after killing and washed several times in warm transport buffer solution until the washing solution remained clear. Subsequently, mucosal layers were manually stripped off the muscle layer, cut into pieces of approximately 4 × 4 cm and stored in transport buffer solution at 38 °C until mounting.

2.4. Incubation

Epithelia were mounted in Ussing chambers with an exposed area of 3.14 cm^2. We used silicon rubber rings on both sides of the epithelia to minimize edge damage. Chambers were connected to reservoirs containing 15 ml incubation buffer solution on each side. Solutions were kept at 38 °C by thermostated water jackets and continuously stirred by use of a gas lift system. The time between removal of epithelia and mounting into Ussing chambers did not exceed 45 min.

2.5. Electrophysiological measurements

Ussing chambers were connected to a computer-controlled voltage clamp device (Ing.-Büro für Mess- und Datentechnik, Aachen, Germany). Transepithelial potential difference (V_t) was measured through KCl agar bridges and Ag-AgCl electrodes. Short bipolar current impulses (100 μA for 300 ms) were applied by a second pair of electrodes connected via NaCl agar bridges. Impulse-induced changes of V_t were measured to calculate tissue conductance ($G_t = \delta I/\delta V_t$). The second pair of electrodes also served for continuous application of an external current to clamp V_t to 0 mV (short-circuit), − 40 mV or + 40 mV. Under short-circuit conditions, the clamp current equates the current across the epithelium induced by active charge transfer (short-circuit current, I_{sc}) but is oppositely directed. Fluid resistance and junction potentials were measured before mounting the epithelia. The values obtained were used to correct clamp currents and electrophysiological data.

Ouabain (0.1 mmol l^{-1}) was used to inhibit the Na^+-K^+ ATPase (Valente et al., 2003). The involvement of an anion channel in acetate transport was examined by pre-incubating epithelia with the anion channel blocker DIDS (1 mmol l^{-1}) (Stumpff et al., 2009; Georgi et al., 2013). Ouabain was added to the serosal side of epithelia exclusively; DIDS was used on the same side where acetate was added later on. Preincubation with blocker substances lasted 30 (DIDS) or 60 min (ouabain), respectively. Thereafter, the concentration of acetate was raised from 0 to 40 mmol l^{-1} on either the mucosal or the serosal side by partial buffer exchange (i.e. substitution of 7 ml incubation buffer by 7 ml acetate-rich buffer solution). Attenuation of blocker concentration due to buffer exchange was prevented by addition of the appropriate amount of the respective inhibitor. Undulations of hydrostatic pressure during partial buffer exchange were prevented by disabling the buffer circulation between the Ussing chamber halves and their above buffer reservoirs with arterial forceps.

2.6. Flux studies

Epithelia were incubated under short-circuit conditions with control buffer solution containing acetate at 40 mmol l^{-1} on both the mucosal and serosal sides. Approximately 20 min after mounting, epithelia were paired according to similarity in G_t (i.e. difference between corresponding epithelia < 25%). One epithelium of each pair received 70 kBq of ^{14}C–acetate on the mucosal side, whereas the corresponding epithelium received the same amount of radioactively labelled acetate on the serosal side ("hot" sides). Three consecutive flux periods of 60 min duration started 45 min after addition of radioactivity. The V_t was clamped to − 40, 0 and + 40 mV during the flux periods, and the order of V_t was alternated between experiments to avoid bias by time effects. Mucosal-to-serosal (ms) and serosal-to-mucosal (sm) flux rates of acetate (J_{ms}^{Ac}, J_{sm}^{Ac}) were calculated by relating the radioactivity appearing on the unlabelled (cold) side to the specific activity on the labelled (hot) side. Radioactivity was measured in a liquid scintillation