Transport of ketone bodies and lactate in the sheep ruminal epithelium by monocarboxylate transporter 1

FRANK MÜLLER,1 KORINNA HUBER,2 HELGA PFANNKUCHE,3 JÖRG R. ASCHENBACH,1 GERHARD BREVES,2 AND GOTTHOLD GÄBEL1
1Veterinär-Physiologisches Institut, Universität Leipzig, D-04103 Leipzig; and 2Physiologisches Institut, Tierärztliche Hochschule Hannover, D-30173 Hannover, Germany

Received 19 June 2001; accepted in final form 23 May 2002

Müller, Frank, Korinna Huber, Helga Pfannkuche, Jörg R. Aschenbach, Gerhard Breves, and Gotthold Gäbel. Transport of ketone bodies and lactate in the sheep ruminal epithelium by monocarboxylate transporter 1. Am J Physiol Gastrointest Liver Physiol 283: G1139–G1146, 2002. First published May 29, 2002; 10.1152/ajpgi.00268.2001.—Due to intensive intracellular metabolism of short-chain fatty acids, ruminal epithelial cells generate large amounts of d-β-hydroxybutyric acid, acetoacetic acid, and lactic acid. These acids have to be extruded from the cytosol to avoid disturbances of intracellular pH (pHi). To evaluate acid extrusion, pH was studied in cultured ruminal epithelial cells of sheep using the pH-sensitive fluorescent dye 2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein. Extracellular addition of d-β-hydroxybutyrate, acetoacetate, or lactate (20 mM) resulted in intracellular acidification. Vice versa, removing extracellular d-β-hydroxybutyrate, acetoacetate, or lactate after preincubation with the respective monocarboxylate induced an increase of pHi. Initial rate of pH decrease as well as of pH recovery was strongly inhibited by pCMBS (400 μM) and phloretin (20 μM). Both cultured cells and intact ruminal epithelium were tested for the possible presence of proton-linked monocarboxylate transporter (MCT) on both the mRNA and protein levels. With the use of RT-PCR, mRNA encoding for MCT1 isoform was demonstrated in cultured ruminal epithelial cells and the ruminal epithelium. Immunostaining with MCT1 antibodies intensively labeled cultured ruminal epithelial cells and cells located in the stratum basale of the ruminal epithelium. In conclusion, our data indicate that MCT1 is expressed in the stratum basale of the ruminal epithelium and may function as a main mechanism for removing ketone bodies and lactate together with H+ from the cytosol into the blood.

intracellular pH; short-chain fatty acids; intracellular metabolism


The rumen is the greatest compartment of the ruminant forestomach. In this compartment, large amounts of short-chain fatty acids (SCFA; ~5 M·day⁻¹·sheep⁻¹; mainly acetic acid, propionic acid, and n-butyric acid) are synthesized by microbial fermentation of carbohydrates (1). SCFA are directly absorbed from the rumen due to effective uptake by the ruminal epithelial cells (9, 14, 15).

After luminal uptake, a high portion of SCFA is metabolized intracellularly (1, 2, 21). Intracellular SCFA metabolism is vital to cover the huge energy demand of the ruminal epithelium (4, 21). The end products of epithelial SCFA metabolism are ketone bodies (d-β-hydroxybutyric acid and acetoacetic acid) and lactic acid. Ketone bodies and lactate need to be efficiently extruded from the ruminal epithelial cells to 1) prevent a lethal drop of intracellular pH (pHi) and 2) counteract osmotic load of the cytosol. However, d-β-hydroxybutyric acid, acetoacetic acid, and lactic acid are even less lipophilic than SCFA in their undissociated form (18). Therefore, the undissociated forms of ketone bodies and lactate cannot cross the membrane well by free diffusion. Additionally, d-β-hydroxybutyric acid, acetoacetic acid, and lactic acid (pK = 4.4, 3.6, 3.8, respectively) are almost completely dissociated at physiological pH (~7.37) (19), implying that membrane permeation by free diffusion is very low. Consequently, a specific transport mechanism for rapid export of ketone bodies and lactate seems to be required in the membrane of ruminal epithelial cells.

Existence of such a transport system may be derived from studies in portal-drained viscera of sheep and cattle (15, 16, 21, 22). In these studies, an increase of intraruminal n-butyrate concentration led to an increased appearance of ketone bodies in the blood, suggesting a directed export of ketone bodies across the basolateral membrane. However, possible export mechanisms have not yet been investigated.

A candidate system for ruminal monocarboxylate transport is the proton-linked monocarboxylate transporter (MCT). In a variety of mammalian cell types, several MCT isoforms have been identified and characterized to transport monocarboxylates such as lactate, β-hydroxybutyrate, and acetoacetate together with protons (12). Among the MCT isoforms, MCT1 has been investigated in the most detail and was found to be ubiquitously expressed (12). Studies on epithelial cells of the gastrointestinal tract suggested that only this isoform plays a major role in the transport of various monocarboxylates, whereas other MCT isoforms seem to be of little or no importance (11, 12, 20, 23, 24).

THE RUMEN IS THE GREATEST compartment of the ruminant forestomach.

Address for reprint requests and other correspondence: H. Pfannkuche, Veterinär-Physiologisches Institut, Universität Leipzig, An den Tierkliniken 7, D-04103 Leipzig, Germany (E-mail: pfannku@rz.uni-leipzig.de).

http://www.ajpgi.org 0193-1857/02 $5.00 Copyright © 2002 the American Physiological Society G1139
The first aim of the present study was to elucidate the ability of the ruminal epithelium to transport ketone bodies and lactate. Ruminal epithelial cells were isolated and cultured to measure monocarboxylate-dependent changes of pH. Decrease or recovery of pH was used to assess transport processes of ketone bodies and lactate across the cell membrane. Because pH studies pointed to MCT1 as the main carrier for these monocarboxylates, MCT1 expression was examined on mRNA as well as protein levels in both ruminal epithelium and cultured ruminal epithelial cells. Immunodetection of MCT1 was further used to identify the localization of the transporter within the multilayered squamous epithelium of the sheep rumen.

METHODS

Animals

Adult Merino sheep were used for all experiments. The animals had been fed with hay ad libitum. They were slaughtered according to the good manufacturing practice standards of meat production, i.e., stunning by a captive-bolt pistol and killing by exsanguination in the faculty abattoir.

Measurement of pH

Primary culture of ruminal epithelial cells of sheep. The experimental protocols for cultivation of sheep ruminal epithelial cells as well as measurement of pH have been described previously (19). In brief, after exsanguination and exenteration, papillae were excised from the atrium ruminis. Thereafter, cells were separated from the papillae by fractional trypsinization. Isolated cells were suspended in medium 199 (M-199) supplemented with 15% FBS, 20 mM HEPES, 50 µg/ml gentamicin, and 2 mM l-glutamine and seeded onto round glass coverslips (10 mm diameter; Chance Propper) placed in plastic tissue culture dishes (35 mm diameter; Corning) or onto cell culture bottoms and frozen in liquid nitrogen. To isolate poly(A) RNA from the ruminal epithelium, pieces of sheep ruminal wall were cut out 2–4 min after slaughter. Thereafter, ruminal epithelia were isolated by removing serosa and muscle layers as described by Gäbel et al. (10) and frozen in liquid nitrogen. Total RNA was isolated from homogenized cells or tissues by chloroform/phenol extraction according to the manufacturer’s protocol (RNA Isolation Kit, Stratagene, La Jolla, CA). Poly(A)+ RNA from cultured ruminal epithelial cells or ruminal epithelium was enriched by affinity chromatography using oligo(dT) cellulose.

RT-PCR

Cultured ruminal epithelial cells were scraped from the bottom of culture flasks and frozen in liquid nitrogen. To isolate poly(A)+ RNA from the ruminal epithelium, pieces of sheep ruminal wall were cut out 2–4 min after slaughter. Thereafter, ruminal epithelia were isolated by removing serosa and muscle layers as described by Gäbel et al. (10) and frozen in liquid nitrogen. Total RNA was isolated from homogenized cells or tissues by chloroform/phenol extraction according to the manufacturer’s protocol (RNA Isolation Kit, Stratagene, La Jolla, CA). Poly(A)+ RNA from cultured ruminal epithelial cells or ruminal epithelium was enriched by affinity chromatography using oligo(dT) cellulose.

First-strand synthesis was performed using Moloney murine leukemia virus (MMLV) RT (Amersham). Poly(A)+ RNA (5 µg) of cultured ruminal epithelial cells or the sheep ruminal epithelium was transcribed into cDNA by 500 U of MMLV RT with 250 pmol oligo(dT) primer (Amersham Pharmacia Biotech) following the manufacturer’s instructions. MCT1-specific PCR primers were derived from human MCT1 mRNA sequence (synthesis: Roth, Germany): sense primer 5’-gggagaggctctcattga-3’ coding for the initial part of transmembrane region TM6, antisense primer 5’-ccaaagccaggtgatgatg-3’ coding for the initial part of transmembrane region TM2. The reaction conditions were as follows: 1× reaction buffer (200 mM Tris-HCl, pH 8.4; 500 mM KCl), 0.4 mM each of 2-deoxynucleotide 5’-triphosphate, 1.5 mM MgCl2, 2.5 U Taq polymerase (GIBCO-BRL), 20 pmol of each primer, and 2 µl of template; the total reaction volume was 25 µl. PCR was performed in a Mastercycler Gradient (Eppendorf) for 30 cycles, each one comprising the denaturation for 30 s at 94°C, the annealing for 1 min at 58°C, and the elongation for 2 min at 72°C. Finally, a prolonged elongation was given at 72°C for 15 min. PCR products were separated by agarose gel electrophoresis (1% agarose). Ethidium bromide-stained gel showed one separate band at ≈750 bp for ruminal epithelial cells and ruminal epithelium, respectively. The band was cut out, and pure cDNA was isolated using JetSorb Kit (Genomed). The cDNA fragment of cultured ruminal epithelial cells or the ruminal epithelium was cloned in the vector pGEM-T (Promega) by Taq-polymerase-amplified (T/A) cloning using a T4 DNA ligase. Nucleic acid sequence of inserts was determined by a commercial laboratory (TOPLAB).
**Immunohistochemistry**

Immunohistochemical labeling was performed on cultured ruminal epithelial cells grown on coverslips and on cryostat sections of the ruminal epithelium. Cultured cells and full thickness specimens of the left lateral ruminal wall (for cryostat sections) were fixed for 24 h in 0.1 M phosphate buffer containing 4% formaldehyde and 0.2% picric acid. After being fixed, cultured cells and tissues were washed with 0.1 M phosphate buffer (3 × 10 min) and stored in PBS containing 0.1% NaN₃. For the cryostat sections, the tissue was prepared by overnight incubation at 4°C in PBS containing 30% sucrose. Transversal sections (15 μm) were cut using a cryostat and mounted on poly-L-lysine-coated slides.

Cultured cells and sections were processed for fluorescence immunohistochemistry as described in detail previously (25). Briefly, after being washed and preincubated for 60 min in PBS (G/TX) solution (PBS containing 4% goat serum and 0.5% Triton X-100), cultured cells and tissues were incubated with combinations of the primary antibodies for 12–16 h at room temperature. A primary antibody against MCT1 (chicken-anti-MCT1; AB1286; Chemicon) was used in a dilution of 1:300. Control experiments were carried out by preincubating the antibody in the presence of 10 μM of the peptide antigen (Biotrend) according to the COOH-terminal region of rat MCT1 (LQNSSGDPAEEESPV) for 24 h at 4°C before use.

To differentiate between epithelium and subepithelial layers, the basal lamina was stained by using a primary antibody against laminin (mouse-anti-laminin; 1087746; Roche Diagnostics) in a dilution of 1:500. Both cultured cells and epithelial preparations were washed three times for 10 min after application of the primary antibodies. Thereafter, cultured cells or epithelial preparations were incubated in PBS (G/TX) solution containing secondary anti-chicken or anti-mouse IgG raised in goats and conjugated to FITC or Cy3 (Dianova). Secondary antibodies were diluted 1:100 (anti-chicken FITC) and 1:500 (anti-mouse Cy3), respectively. After the final washing, cultured cells on coverslips and epithelial preparations were mounted on slides in a PBS/glycerol solution and analyzed using an epifluorescence microscope (IX70, Olympus) attached to an image-analysis system (video camera: model 4910, Cohu; Macintosh computer and IPLab Spectrum 3.0 software, Signal Analytics). To prove specificity of secondary antisera, they were applied without use of primary antibody. No staining was seen after omitting the primary antibodies (not shown).

**Chemicals**

BCECF/AM was obtained from Calbiochem. Acetoacetate was purchased from AppliChem. Unless mentioned otherwise, all other chemicals were of highest analytical grade and obtained from either Sigma-Aldrich or Merck.

**Statistics**

Results are expressed as arithmetic means ± SE. pHᵢ per minute are changes in pHᵢ during 1 min of cell incubation with the new medium. Differences between two means were tested using Student’s t-test, paired or unpaired as appropriate (normal distribution), or Mann-Whitney rank sum test (no normal distribution). ANOVA was applied to compare multiple means. If this indicated a significant difference between means, the Student-Newman-Keuls test was applied to determine which of the means differed from each other. Statistical tests were performed using SigmaStat 2.0 (SPSS Science). Results were regarded to be significantly different at P < 0.05.

**RESULTS**

**Effects of Extracellular Monocarboxylates on pHᵢ in Ruminal Epithelial Cells**

The effect of 20 mM D/L-lactate (approximately equal amounts of D and L isomers) on intracellular pH was determined in cultured ruminal epithelial cells. Addition of D/L-lactate produced a rapid decrease in pHᵢ (Fig. 1).

To examine whether lactate decreased pHᵢ via proton-linked monocarboxylate transport, we tested the effects of pCMBS and phloretin, ubiquitously used and well-established inhibitors of MCT (5, 10, 11). As shown in Fig. 1, both inhibitors decreased the initial rate of intracellular acidification (pHᵢ/min, i.e., change of pHᵢ during 1 min after incubation in the new medium). Compared with control, presence of pCMBS (400 μM) reduced the initial rate of intracellular acidifica-
tion by 83% (−0.18 ± 0.04 vs. −0.03 ± 0.01 pH U/min; 
P < 0.01; Fig. 1A). Phloretin (20 μM) inhibited rate of
intracellular acidification by 78% (−0.18 ± 0.04 vs.
−0.04 ± 0.01 pH U/min; P < 0.01; Fig. 1B).
To assess whether ketone bodies can also enter cells
by the carrier-mediated process, we investigated the
effects of d-β-hydroxybutyrate and acetoacetate on pH,
compared with the effect of l-lactate. When cells were
exposed to 20 mM d-β-hydroxybutyrate or 20 mM ace-
toacetate, a fast decrease in pH occurred that was
similar to that produced by equimolar amounts of l-
lactate (Fig. 4).
As shown in Fig. 2A, application of pCMBS (400 μM)
inhibited the initial rate of intracellular acidification
following the addition of d-β-hydroxybutyrate by 75%
(−0.20 ± 0.02 vs. −0.05 ± 0.02 pH U/min; 
P < 0.05;
Fig. 2A). Presence of pCMBS diminished the initial
rate of acetoacetate-induced acidification by 81%
(−0.16 ± 0.01 vs. −0.03 ± 0.03 pH U/min; 
P < 0.05;
Fig. 2B). The initial rate of L-lactate-induced acidification
was reduced by 64% in the presence of pCMBS
(−0.19 ± 0.03 vs. −0.07 ± 0.00 pH U/min; 
P < 0.05;
Fig. 2C).

*pH* Recovery from Monocarboxylates-Induced
Cell Acidification

The data presented above suggest that d-β-hydroxy-
butyrate, acetoacetate, and l-lactate can be taken up
by a proton-linked MCT. Under physiological condi-
tions, however, intracellular but not extracellular pres-
ence of these monocarboxylic acids represents the ma-
jor challenge for the ruminal epithelial cells (see
introduction). Thus rapid extrusion of monocarboxy-
lates and protons is of greater physiological importance
than uptake. Therefore, we loaded cells with monocar-
boxylic acids first and examined recovery of pH after
extracellular removal of the acids.

Figure 3 shows that removal of extracellular d/L-
lactate, following preincubation with 20 mM d/L-lac-
tate, resulted in a rapid recovery of pH. In comparison,
the presence of 400 μM pCMBS (Fig. 3A) and 20 μM
phloretin (Fig. 3B) reduced the initial rate of pH re-
cov ery by 77 (0.13 ± 0.03 vs. 0.02 ± 0.00 pH U/min; 
P <
0.05) and 62% (0.13 ± 0.03 vs. 0.05 ± 0.01 pH U/min;
P < 0.05), respectively.

Similar results were obtained after loading cells with
d-β-hydroxybutyrate, acetoacetate, or l-lactate. As
shown in Fig. 4, a recovery of pH occurred immediately
after removing d-β-hydroxybutyrate (Fig. 4A), acetoac-
etate (Fig. 4B), or l-lactate (Fig. 4C) from the incuba-
tion solution.

In the presence of pCMBS (400 μM), the initial rate
of pH recovery was reduced by 73% (0.15 ± 0.03 vs.
0.04 ± 0.03 pH U/min; 
P < 0.05), by 81% (0.16 ± 0.02
vs. 0.03 ± 0.01 pH U/min; 
P < 0.05), and by 80%
(0.15 ± 0.02 vs. 0.03 ± 0.01 pH U/min; 
P < 0.05) for
d-β-hydroxybutyrate, acetoacetate, and l-lactate, re-
spectively (Fig. 4, A-C).

Detection of MCT1 by RT-PCR

With the use of specific primers derived from human
MCT1 sequence, a 753-bp cDNA fragment was ob-
tained by RT-PCR amplification of poly(A)+ RNA from
both cultured ruminal epithelial cells and the ruminal
epithelium (Fig. 5).
Specificity of the PCR products was confirmed by
sequencing. Deduced amino acid sequences of the am-
plified MCT1 cDNA fragments from both cultured cells
and the epithelium were identical. The sequence showed 83–84% homology to the analogous region of rat, hamster, and human MCT1 (GenBank accession nos. D63834, L25842, and NM-003051; Fig. 5).

Detection and Localization of MCT1 Protein by Immunohistochemistry

Antilaminin staining resulted in labeling of the basal lamina and the connective tissue of the papillae (Fig. 6A). Labeling of the epithelial basal lamina was used to clearly identify localization of MCT1 immunoreactive cells. MCT1 immunoreactivity was restricted to an only small cell fraction of the stratified ruminal epithelium. These cells were localized close to the antilaminin-stained basal lamina, i.e., they belonged to the stratum basale (Fig. 6B).

Similar to the epithelial preparations, MCT1 immunoreactivity was also detectable in cultured ruminal epithelial cells. MCT1 protein could be demonstrated in the vast majority of cultured cells. MCT1-positive staining was distributed over the whole cell area (Fig. 6D).

Preabsorption with the specific peptide antigen abolished MCT1-specific staining completely, both in the ruminal epithelium (Fig. 6C) and in cultured ruminal epithelial cells (Fig. 6E).

DISCUSSION

In the ruminal epithelium, apical uptake and intracellular metabolism of SCFA release large amounts of D-β-hydroxybutyric acid, acetoacetic acid, and lactic acid. The extrusion of these acid energy substrates into the
MCT1 IN THE SHEEP RUMINAL EPITHELIUM

Fig. 5. Detection of a MCT1-specific cDNA fragment by RT-PCR from the sheep ruminal epithelium and cultured ruminal epithelial cells. RT-PCR analysis was carried out using monocarboxylate transporter (MCT) 1-specific primers derived from human MCT1 mRNA sequence. PCR fragments were isolated in a 1% agarose gel. Lanes b and d: MCT1 fragment of sheep ruminal epithelium (b) and cultured ruminal epithelial cells (d). Lanes a and c: RT-PCR was performed without RT (−RT) to exclude genomic DNA contaminations in the epithelium (a) and in cultured cells (c). The arrow indicates the band corresponding to the expected 753-bp cDNA fragment [DNA ladder (M)]. Deduced amino acid sequence of the cDNA fragments is shown at the bottom (in comparison with the analogous region of rat, hamster, and human MCT1). Positions that differed in 2 or more MCT1 sequences are boxed.

Identification of MCT

The proton-linked MCT was structurally detected on the molecular level by RT-PCR. Nine MCT-related sequences have been identified in mammalian cells so far (12), but only MCT1–4 have been expressed in an active form and characterized as proton-linked MCTs (12). Of these, solely the MCT1 isoform has been demonstrated to mediate proton-linked monocarboxylate transport in other gastrointestinal epithelia (11, 20, 24). With the use of specific primers derived from human MCT1 sequence, PCR products of expected length (753 bp) were obtained by amplification of poly(A)+ RNA isolated from either the cultured epithelium or the sheep ruminal epithelium (Fig. 5). The deduced amino acid sequences of the 753-bp cDNA fragments showed ~85% homology with the analogous regions of rat, hamster, and human MCT1, respectively. The high sequence homology revealed the presence of mRNA coding for MCT1 isoform in both the cultured cells and the ruminal epithelium. The expression of the corresponding MCT1 protein was confirmed by specific MCT1 immunostaining with an anti-MCT1 antibody (Fig. 6). The detection of specific MCT1 expression in mucosal cells of the sheep ruminal epithelium is a prerequisite for maintaining the functional integrity of the ruminal epithelium and for the energy supply to the animal. The present study indicates that an MCT1 located in the stratum basale may play a key role in this process. Its functional characteristics were studied using cultured ruminal epithelial cells. Structural evidence for MCT1 expression was demonstrated on mRNA as well as protein level in the ruminal epithelium and in cultured epithelial cells.

pH Regulation and Monocarboxylate Transfer by MCT

Linkage between proton transfer and transfer of monocarboxylates was detectable during both uptake and extrusion. Extracellular addition of d-α-hydroxybutyrate, acetooacetate, or d/L-lactate caused intracellular acidification resulting in a lower steady-state pH (Figs. 1 and 2). Vice versa, removal of the respective monocarboxylates from the buffer solutions resulted in a recovery from the decreased steady-state pH (Figs. 3 and 4). However, these pH changes may be induced by either nonionic diffusion of protonated monocarboxylates or by a carrier-mediated process. Because both the protonated and the dissociated forms of ketone bodies and lactate can hardly cross the plasma membrane by free diffusion (18), existence of a carrier-based transfer of monocarboxylate anions and protons can be postulated from a teleological point of view.

In a variety of other cell types studied so far, transport of monocarboxylates across the membranes is mediated by proton-linked MCTs (12). Various inhibitors have been shown to block MCTs in these cells (3, 11–13, 24). Of these, pCMBS, a noncompetitive blocker of MCT1 isoform (11), and phloretin were applied in the present study. Presence of pCMBS significantly inhibited the rate of intracellular acidification induced by the extracellular addition of monocarboxylates as well as the pH recovery after removing the monocarboxylates. The degree of inhibition by phloretin (tested on d/L-lactate effects only) was similar to the degree of inhibition by pCMBS. These functional data indicate that transport of ketone bodies and lactic acid is mediated by a proton-linked MCT. Ketone bodies are transported only by MCT1 and/or MCT2 in various cell types (12). The most striking biochemical difference between these two isoforms is the different sensitivity to pCMBS. MCT1 but not MCT2 is sensitive to pCMBS (11, 12). Consequently, pH studies suggest that MCT1 is predominantly involved in transmembrane transfer of ketone bodies and lactate.
mRNA as well as of the MCT1 protein in both ruminal epithelium and cultured ruminal epithelial cells strongly supports the hypothesis that transport of ketone bodies and lactate is predominantly mediated by MCT1.

Although the expression of MCT1 isoform was clearly demonstrated, it should be emphasized that other isoforms of MCT may additionally be present. But despite the putative presence of other isoforms, they probably play an inferior role in the permeation of ketone bodies and lactate. The pCMBS sensitivity of monocarboxylate transport showed by pH_i studies (Figs. 2 and 4) suggested that mainly MCT1 mediates the transport of ketone bodies and lactate. Therefore, other MCT isoforms, even if expressed, do not seem to be important for the transport of relevant monocarboxylates in the ruminal epithelium. Similar to our findings, studies in nonruminant species (11, 20, 24) indicate that MCT1 plays a central role in intestinal transport of monocarboxylates. Nevertheless, expression of other MCTs and a supporting role in ruminal monocarboxylate transport cannot totally be exclude.

Localization of MCT1

The ruminal epithelium is a stratified squamous epithelium consisting of the stratum basale, the stratum spinosum, the stratum granulosum, and the stratum corneum. The total number of cell layers varies between 10 and 35 (depending on diet) (7). To detect the localization of MCT1 within the tissue, immunofluorescence studies using an anti-MCT1 antibody were performed. MCT1 immunoreactivity was expressed only in cells of the stratum basale (Fig. 6A). Localization of MCT1 in the cell layer directed to the blood side of the ruminal epithelium is in line with in vivo studies in portal-drained viscera (15, 21). In these studies, the ruminal epithelium showed a strong prevalence to release ketone bodies and lactate on the serosal side, i.e., into the blood.

The visualization of MCT1 immunoreactivity in the basal layer (Fig. 6B) may also contribute to understanding the functional organization of the ruminal epithelium. It is still unknown where the transporting proteins are located in the multilayered structure of the ruminal epithelium (8, 9, 17, 26) and how the polarization of the epithelium is realized. In this context, our results could provide a hint, how the term “basolateral” (according to the nomenclature used in monolayered epithelia) could be interpreted in the multilayered ruminal epithelium, at least for the expression of MCT1 (Fig. 6B), i.e., the directed transport of ketone bodies and lactate.

MCT1 immunoreactivity was also detected in cultured ruminal epithelial cells. In contrast to the intact epithelium, MCT1 protein could be detected in the vast majority of cultured cells. Additionally, it was found that the MCT1-positive staining was distributed over the whole cell area (Fig. 6D). The latter finding supports the earlier view that ruminal epithelial cells do not polarize in culture (6). However, the presence of mRNA coding for MCT1 and the expression of MCT1 protein in both the cultured cells and the cells of the stratum basale of the ruminal epithelium indicate a conserved expression of this transport protein during cultivation. Thus cultured cells are molecularly similar to ruminal epithelial cells of the stratum basale, at least as regards expression of MCT1.

In conclusion, the present study demonstrates that MCT1 is expressed in the stratum basale of the ruminal epithelium and that it seems to play a major role in the extrusion of ketone bodies and lactate from the ruminal epithelium. Besides the ruminal $\text{Na}^+/\text{H}^+$ ex-
chenger and the bicarbonate transporting system(s) (19), MCT1 expression is important for maintaining homeostasis of pH$_3$ and thus an undisturbed intracellular metabolism. Because ketone bodies and lactate are osmotically active, MCT1 has also to be regarded as a component for maintaining intracellular osmolality. Moreover, the basal layer localization of MCT1 facilitates the directional release of ketone bodies and lactate into the bloodstream. Thereby, the ruminal epithelium functions as a two-step system for the absorption of SCFA. In a first step, SCFA are absorbed along a concentration gradient across the apical membrane followed by intraepithelial metabolism to ketone bodies and lactate (1, 2, 21). In a second step, ketone bodies and lactate are transported along their concentration gradients into the blood via MCT1. Consequently, MCT1 plays an indirect but important role for the absorption of SCFA from the forestomach.

We thank C. Koblenz for expert technical assistance in performing the culture of ruminal epithelial cells.

This work was supported by the Deutsche Forschungsgemeinschaft (Ga 329/4–1).

REFERENCES


AJP-Gastrointest Liver Physiol • VOL 283 • NOVEMBER 2002 • www.ajpgi.org

Downloaded from http://ajpgi.physiology.org/ by [10.220.33.4] on July 7, 2017