Functional and molecular biological evidence of SGLT-1 in the ruminal epithelium of sheep

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Received 2 June 1999; accepted in final form 28 January 2000

Because of the high catabolic activity of ruminal microflora, D-glucose is only an intermediate substrate, and its concentration within the rumen is usually very low (≈0.7 mM; Ref. 14). Glucose absorption from the rumen has, therefore, been regarded as of no or only minor importance (2, 19). However, some observations question the standard paradigm for ruminal carbohydrate metabolism. In a clinical study testing the effects of ingesting large quantities of easily fermentable carbohydrates, the D-glucose concentration rose to 10 mM in the forestomach but fell sharply to <0.1 mM in the abomasum, the compartment distal to the forestomach (8). In early studies using the miniature rumen, Tsuda (24) observed that D-glucose disappeared from artificial rumen fluid even against a twofold concentration gradient.

These findings support the suggestion that the ruminal epithelium may have evolved the ability to absorb D-glucose from the lumen to the blood despite the microbial pattern of digestion. The present study was therefore conducted to test the potential for D-glucose absorption and to investigate the underlying transport mechanisms in the ruminal epithelium of sheep. The characterization of transport mechanisms was based on the hypothesis that ruminal glucose transport is similar to the transport process that occurs in the mucosa of the small intestine. Transepithelial D-glucose transport in the intestine is mediated by two separate transport systems operating in series (5). At the mucosal boundary of the epithelial cell, cellular accumulation of sugar occurs against a concentration gradient via electrogenic Na⁺-glucose cotransporter 1 (SGLT-1). At the serosal boundary, a facilitated diffusion transfer system (GLUT-2) allows the accumulated solute to reach the circulating system. The present study reveals that the characteristics of ruminal transport systems for D-glucose closely resemble those of the intestine.

IN RUMINATING ANIMALS like cattle, sheep, and goats, the rumen is the greatest part of the forestomach compartments. The forestomach is a huge fermentation chamber (23–36% of body wt of adult ruminants; Ref. 10) in which ingested carbohydrates are almost completely broken down by symbiotic microorganisms. The major end products of fermentation are the short-chain fatty acids (SCFA) acetate, propionate, and butyrate, and the gases carbon dioxide, methane and H₂ (4).
MATERIALS AND METHODS

Preparation of ruminal epithelium. Sheep (Ovis aries) were fed 100 g of concentrates/day and had ad libitum access to hay and water for at least 2 weeks. The animals were killed by exsanguination after stunning, and the reticulorumen was removed from the abdominal cavity 3–10 min later. A piece (150 cm²) of the ventral rumen was cut out of the ruminal wall and carefully washed in a buffer solution kept at 38°C (for composition of buffer see Solutions). Epithelia were prepared and mounted in Ussing chambers as described by Gabel et al. (7). Experimental procedures were approved by the Regierungspräsidium Leipzig (TVV-No. 13/97).

Electrical measurements. Before mounting of epithelia, junction potential and fluid resistance were determined by a computer-controlled voltage clamp device (Ing.-Büro für Mess- und Datentechnik, Aachen, Germany) for later automatic correction of electrophysiological measurements. Epithelia were short-circuited and were exposed to bipolar impulses of 100 μA for 300 ms at regular intervals (60 s); the subsequently induced changes in transepithelial potential difference and the impulse amplitude were used for calculation of tissue conductance.

Determination of 3-OMG fluxes. All flux measurements were carried out under short-circuit conditions. After mounting, 150 kBq of [3H]-3-OMG were added to the mucosal or serosal side and epithelia were allowed to adapt to experimental conditions for 30 min, i.e., all fluxes were determined under steady-state conditions. Pairs of epithelial sheets matching in conductance (difference <25%) were used for measurement of unidirectional fluxes. Mucosal-to-serosal (J_{m-s}^{3-OMG}) and serosal-to-mucosal (J_{s-m}^{3-OMG}) fluxes of 3-OMG were calculated on the basis of radioactivity appearing at the unlabeled side according to Gabel et al. (7). Radioactivity was determined by scintillation counting (Wallac 1419 LSC, Berthold, Germany) after addition of scintillation fluid (Aquasafe 7300 Plus, Zinsser, Germany) to the samples.

Solutions. The standard buffer solution used for Ussing chamber experiments contained (in mM) 75 NaCl, 25 NaHCO₃, 5 KCl, 2 NaH₂PO₄, 1 Na₃HPO₄, 1 CaCl₂, 2 MgCl₂, 8 NaOH, 5 MOPS, 30 sodium gluconate, and 10 n-butyric acid, gassed with 95% O₂-5% CO₂ (Messer-Griesheim). The buffer solution used for determination of phlorizin sensitivity of 3-OMG fluxes (see Fig. 1) was composed similarly except that 3 mM 3-OMG was added to the solution. In the experiments shown in Fig. 2, 3-OMG was added until the indicated concentration was reached. To determine sodium dependence of 3-OMG-fluxes (see Fig. 3), Na⁺ salts were equimolarly replaced by choline salts. To determine the effects of d-glucose, d-mannose, or 3-OMG on the short-circuit current (Isc) (see Figs. 4–8), epithelia were initially incubated for 3 h in a solution containing 10 mM d-glucose. Subsequently, the d-glucose-containing solution was replaced by a d-glucose-free solution. Further additions are indicated in individual figure legends. When d-glucose, d-mannose, or 3-OMG was added to one side of the tissue, equal amounts of d-mannitol were added to the opposite side to prevent osmotic gradients. In the control groups, equimolar amounts of d-mannitol were added to both sides of the tissues. Initial osmolality of all solutions was determined by freezing point depression (Knauer Osmometer) and adjusted to 288 ± 3 mosmol/kg by adding d-mannitol. Initial pH was adjusted to 7.38 ± 0.03.

Molecular biological methods. Immediately after slaughter (see Preparation of ruminal epithelium), epithelial pieces of the atrium ruminis were carefully washed four times in sterile, ice-cold Dulbecco’s phosphate-buffered saline. Thereafter, ruminal papillae were cut off and shock-frozen in liquid N₂. Frozen papillae were ground with a mortar under N₂ application and care was taken to avoid thawing. From 20 mg of powdered papillae, mRNA was isolated and captured with biotinylated oligo(dT) in streptavidin-coated PCR tubes, using a commercial mRNA capture kit (Boehringer Mannheim). RT-PCR was performed on a Peltier Thermal Cycler 200 (MJ Research, Watertown, MA) using Titan One Tube RT-PCR system (Boehringer Mannheim) and primers (NAPS, Göttingen, Germany) corresponding to bases 187–205 (forward) and 601–621 (reverse) of a mRNA fragment that encodes SGLT-1 in the sheep intestine (GenBank X82410). Amplified cDNA was visualized in ethidium bromide-stained agarose gel (2%) and TA cloned into pCR 2.1/E. coli using TOPO TA cloning kit (Invitrogen, Groningen, The Netherlands). After plasmid purification according to the method of Birnboim and Doly (1), identification and characterization of inserts was performed by restriction analysis with EcoRI, BamH I, Bgl II, and Pst I (New England Biolabs, Beverly, MA). The inserts of two independently isolated clones were sequenced with the ALFExpress II DNA Analysis System and ALFexpress T7 sequencing kit (Amersham Pharmacia Biotech, Freiburg, Germany) after plasmid extraction with Qia- gen Plasmid Miniprep Kit (Qiagen, Hilden, Germany).

Chemicals. Unless stated otherwise, chemicals were obtained either from Sigma Chemical (Deisenhofen, Germany) or from Merck (Darmstadt, Germany). [3H]-3-OMG was obtained from DuPont NEN (Bad Homburg, Germany). The inhibitor of SGLT-1, phlorizin, was dissolved in ethanol and then added to the mucosal buffer reservoir. An equal volume of ethanol was added to the respective side in the control group.

Calculations and statistical analysis. 3-OMG net fluxes (J_{net}^{3-OMG}) at different 3-OMG concentrations (see Fig. 2) were fitted to the following equation

\[
J_{net}^{3-OMG} = J_{net}^{3-OMG}(max) \left(\frac{[3-OMG]}{K_{3-OMG}^{0.5} + [3-OMG]}\right)
\]

where \(J_{net}^{3-OMG}(max)\) is the maximal 3-OMG net flux at saturating substrate concentration, [3-OMG] is 3-OMG concentration, and \(K_{3-OMG}^{0.5}\) is the 3-OMG concentration at 0.5 \(J_{net}^{3-OMG}\). By using the same mathematical approach, the maximum increases in \(I_{sc}\) inducible by d-glucose (\(I_{sc}(D-gluc)\)) or by 3-OMG (\(I_{sc}(3-OMG)\)) and the respective substrate affinities were calculated from the increases in \(I_{sc}\) 3 min after mucosal addition of d-glucose or 3-OMG. To determine the significance of differences between two groups, unpaired Student’s t-test was applied. To determine treatment effects within one epithelium, paired Student’s t-test was used. When multiple means were compared, one-way ANOVA was carried out on the data first. If this indicated a significant difference between means, Student-Newman-Keuls test was used to determine which of the means differed from each other. Statistical tests were performed using Sigma-Stat 2.0 software (Jandel, Erkrath, Germany). Data presented are means ± SE.

RESULTS

3-OMG fluxes. To test the ability of the ruminal epithelium for d-glucose transport, we measured the unidirectional transport of 3-OMG, a nonmetabolizable glucose derivative (21). In the small intestine, 3-OMG is accepted by the apically located SGLT-1 as well as by the basolaterally located facilitated transporter (11, 23). The concentration of the glucose derivative was 3 mM on both sides of the epithelium. In the absence of

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an electrochemical gradient, there was significant $J_{\text{z-OMG}}^{\text{net}}$ that decreased after the mucosal addition of 0.1 mM phlorizin (Fig. 1). Sixty minutes after phlorizin addition, $J_{\text{z-OMG}}^{\text{net}}$ was abolished, i.e., $J_{\text{z-OMG}}^{\text{ms}}$ was not significantly different from $J_{\text{z-OMG}}^{\text{sm}}$. The phlorizin-induced decrease in $J_{\text{z-OMG}}^{\text{net}}$ was due solely to a decrease in mucosal-to-serosal flux, whereas the flux in the opposite direction was not affected (Fig. 1).

Further experiments were carried out to assess the dependence of 3-OMG transport on substrate concentration. Figure 2 demonstrates the net transport of 3-OMG as a function of increasing 3-OMG concentrations on both sides of the epithelium. As the concentration of extracellular 3-OMG increased from 0.25 mM to 7 mM, $J_{\text{z-OMG}}^{\text{net}}$ was increased and subsequently became saturated in conformity with Michaelis-Menten kinetics. Nonlinear regression analysis yielded an apparent $K_{0.5}^{\text{z-OMG}}$ of 1.48 ± 0.27 mM and a $J_{\text{z-OMG}}^{\text{net-max}}$ of 23.3 ± 1.4 mmol · cm$^{-2}$ · h$^{-1}$. At all concentrations tested, mucosal addition of 0.1 mM phlorizin completely abolished net transport within 90 min (Fig. 2).

The dependence of 3-OMG transport on sodium was tested by measuring 3-OMG fluxes at concentrations of 10, 40, and 140 mM sodium in the buffer solution. The 3-OMG concentration was kept at 3 mM. Figure 3 shows that decreasing the sodium concentration from 140 to 40 mM had no effect on unidirectional 3-OMG fluxes, whereas a further decrease to 10 mM led to a significant reduction of $J_{\text{z-OMG}}^{\text{net}}$. The decrease in net flux of 3-OMG was solely due to a reduction of mucosal-to-serosal flux. No significant change in serosal-to-mucosal flux was observed.

**Electrogenic effects of mucosal D-glucose.** To evaluate the electrogenicity of the apical glucose transporter, the short-term effects of mucosal D-glucose or 3-OMG on $I_{\text{sc}}$ were investigated. As shown in Fig. 4, the addition of 10 mM D-glucose or 10 mM 3-OMG to the apical bathing solution resulted in a significant and immediate increase in $I_{\text{sc}}$. Within 3 min, D-glucose stimulated $I_{\text{sc}}$ by 0.26 ± 0.02 μeq · cm$^{-2}$ · h$^{-1}$. The increase of $I_{\text{sc}}$ induced by 3-OMG amounted to 0.12 ± 0.02 μeq · cm$^{-2}$ · h$^{-1}$ and was smaller ($P < 0.01$) than the increase induced by D-glucose. Both D-glucose- and 3-OMG-induced stimulation of $I_{\text{sc}}$ were almost com-
pletely abolished by mucosal addition of 0.1 mM phlorizin. (Fig. 4). Addition of phlorizin to control epithelia incubated in D-glucose-free solutions (D-glucose replaced by D-mannitol) had no effect. Within 15 min after D-glucose or 3-OMG addition, the tissue conductance declined from 2.94 ± 0.65 mS/cm² to 2.44 ± 0.64 mS/cm² and from 2.98 ± 0.35 mS/cm² to 2.63 ± 0.34 mS/cm², respectively. The extent of decrease in the both groups was not significantly different (ANOVA) from that observed in the D-mannitol-treated group (from 2.91 ± 0.55 mS/cm² to 2.34 ± 0.34 mS/cm²).

Electrogenic effects of serosal D-glucose, D-mannose, and 3-OMG. In the chicken cecum, an epithelium that functions similar to ruminal epithelium in some respects, addition of D-glucose to the serosal side caused a greater stimulation of $I_{sc}$ than application of D-glucose to the mucosal side, indicating metabolic actions of D-glucose (9). To test whether such indirect metabolic effects of D-glucose are present in the ruminal epithelium, 10 mM D-glucose was added to the serosal side after the epithelia were incubated in D-glucose-free solutions for 30 min. The addition of 10 mM D-glucose on the serosal side also led to an increase in $I_{sc}$ (Fig. 5). However, 3-OMG failed to stimulate $I_{sc}$ when added to the serosal side (Fig. 5).

To further evaluate potential metabolic effects of D-glucose, experiments were conducted in which D-mannose was included in the serosal medium. D-Mannose is a suitable substrate for the basolaterally located Na$^+$-independent transporter but not for the apical Na$^+$-dependent transporter (13), and it can be phosphorylated and converted to fructose-6-phosphate by most cells (17). Similar to D-glucose, addition of 10 mM D-mannose to the serosal side produced a strong increase of $I_{sc}$ (Fig. 6). The subsequent serosal application of D-glucose led to only a delayed, small additional increase of $I_{sc}$ (Fig. 6).

Effect of mucosal D-glucose or 3-OMG after serosal stimulation of $I_{sc}$. To test whether electrogenicity of mucosal glucose uptake is still present after serosal stimulation of $I_{sc}$, 10 mM D-glucose was applied to the mucosal side in the plateau phase after addition of 10 mM D-mannose or 10 mM D-glucose to the serosal side. As shown in Fig. 7, D-glucose still induced a significant from that observed in the D-mannitol-treated group (from 2.91 ± 0.55 mS/cm² to 2.34 ± 0.34 mS/cm²).

Fig. 3. Dependence of unidirectional [mucosal to serosal (ms), serosal to mucosal (sm)] and net fluxes of 3-OMG on extracellular Na$^+$ concentration. Na$^+$ was replaced by choline on both sides of the epithelium. The 3-OMG concentration was 3 mM on both sides of the epithelium. Data represent means ± SE of 6 epithelia from 4 sheep. Different letters denote significant differences of at least $P < 0.05$ (ANOVA and Student-Newman-Keuls test).

Fig. 4. Effect of mucosal addition of 10 mM D-glucose, 3-OMG (A), or D-mannitol (B; control) on short-circuit current ($I_{sc}$). Phlorizin (0.1 mM) was added to the mucosal side in all groups. Data represent means ± SE of 6 epithelia from 3 sheep. #Significant difference (at least $P < 0.05$) from the value at time of mucosal addition of D-glucose or 3-OMG (paired Student’s $t$-test).

Fig. 5. Effect of serosal addition (vertical arrow) of 10 mM D-glucose, 10 mM 3-OMG, or 10 mM D-mannitol (control) on $I_{sc}$. Data represent means ± SE of 10 epithelia from 5 sheep. *Values of the D-glucose-treated group are significantly different (at least $P < 0.05$) from values of D-mannitol- and 3-OMG-treated tissues (ANOVA and Student-Newman-Keuls test).
rise in \( I_{sc} \). Similarly, mucosal addition of 10 mM 3-OMG significantly enhanced \( I_{sc} \) when it was already increased by serosal addition of 10 mM D-mannose (\( n = 8 \); data not shown).

In the experiments shown in Fig. 2, kinetic data were determined on the basis of 3-OMG fluxes, i.e., they were measured under steady-state conditions and may have been covered by influences of the basolateral glucose transporter and/or by intracellular accumulation of 3-OMG. The importance of the basolateral system in modulating net sugar transfer by altering the transmembranal gradient was precisely shown by Kimmich (15). Therefore, the persistence of the electrogenic effect of mucosally added D-glucose or 3-OMG after serosal preincubation with D-mannose was used to evaluate indirectly the kinetic data of apical glucose transport under initial rate conditions. Serosal preincubation with D-mannose was necessary to minimize the suspected metabolic effects of mucosally added D-glucose.

Figure 8 demonstrates that the glucose-induced \( I_{sc} \) (\( \Delta I_{sc} \)) rose with increasing D-glucose concentrations and became saturated in conformity with Michaelis-Menten kinetics. Nonlinear regression analysis yielded an apparent \( K_{0.5}^{\text{D-Glu}} \) of 0.28 ± 0.05 mM and a \( \Delta I_{sc-max}^{\text{D-glu}} \) of 0.130 ± 0.005 \( \mu \text{eq} \cdot \text{cm}^{-2} \cdot \text{h}^{-1} \). Similar experiments using 3-OMG yielded a \( K_{0.5}^{\text{3-OMG}} \) of 1.51 ± 0.23 mM and a \( \Delta I_{sc-max}^{\text{3-OMG}} \) of 0.125 ± 0.006 \( \mu \text{eq} \cdot \text{cm}^{-2} \cdot \text{h}^{-1} \). \( K_{0.5} \) values were significantly different (\( P < 0.01 \)) for 3-OMG and D-glucose.

**Demonstration of mRNA for SGLT-1.** With specific primers for ovine SGLT-1, RT-PCR of mRNA isolated from ruminal papillae produced a cDNA of \( ~435 \text{ bp} \) (Fig. 9). After TA cloning, 13 of 20 clones were not insert bearing. The remaining seven clones contained the 435-bp insert, as evidenced by \( \text{EcoRI} \) insert excision (data not shown). All inserts could be cut by \( \text{PstI} \), \( \text{BamHI} \), and \( \text{BglII} \) into products of specific length, as predicted for ovine SGLT-1 (Fig. 9). Sequencing of two inserts revealed a 100% identity between the cloned cDNA and mRNA fragment 187–621 of ovine intestinal SGLT-1 (GenBank X82410).

**Fig. 6.** Effect of serosal addition (left vertical arrow) of 10 mM D-mannose on \( I_{sc} \). Seventy minutes after serosal addition of D-mannose, 10 mM D-glucose was added to the serosal side (right vertical arrow). The control group received 2 consecutive doses of 10 mM D-mannitol. Data represent means ± SE of 9 epithelia from 3 sheep. *Values of the D-mannose-treated group are significantly different (at least \( P < 0.05 \)) from values of D-mannitol-treated tissues (Student’s \( t \)-test).

**Fig. 7.** Effect of mucosal (mu) addition (right arrow) of 10 mM D-glucose after serosal (se) stimulation of \( I_{sc} \) by 10 mM D-mannose or 10 mM D-glucose (left arrow). The control group received 2 consecutive doses of 10 mM D-mannitol. Data represent means ± SE of 9 epithelia from 3 sheep. #Significant difference (at least \( P < 0.05 \)) from the value at time of mucosal addition of D-glucose (paired Student’s \( t \)-test).

**Fig. 8.** Concentration dependence of the mucosal stimulation of \( I_{sc} \) by D-glucose or 3-OMG in epithelia pretreated with 10 mM D-mannose. According to the protocol shown in Fig. 7, 10 mM D-mannose was first added to the serosal side. Sixty minutes later (during plateau phase), various concentrations of D-glucose or 3-OMG were added to the mucosal side. D-Glucose- or 3-OMG-induced \( I_{sc} \) (\( \Delta I_{sc} \)) was calculated from the increase of \( I_{sc} \) 3 min after mucosal addition of the substrate. The line was drawn using a nonlinear regression-fitting routine (Marquard-Levenberg algorithm) according to the Michaelis-Menten kinetics formula. Respective kinetic data are specified in the text. Data represent means ± SE of 8 epithelia from 4 sheep.
The study clearly demonstrates that the ruminal epithelium of sheep is able to absorb D-glucose in the direction from lumen to blood. Despite marked differences in their histology (stratified, squamous, and cornified vs. one-layered and columnar) and their physiological role (microbial vs. enzymatic digestion), the nature of D-glucose transport seems to be similar in the rumen and in the intestine. The apical entry of the glucose derivate 3-OMG into the cell was shown to be phlorizin sensitive (Fig. 1), saturable (Fig. 2), and sodium coupled (Fig. 3). All these features are analogous to those that occur in the sodium-coupled transport of D-glucose in the small intestine (5, 27) and indicate the presence of a SGLT on the apical side of the ruminal epithelium.

As regards the basolateral exit of D-glucose, an electroneutral facilitated diffusion transfer system (GLUT 2) has been characterized as the main glucose transporting system in the small intestine of many species (5, 16). In the present study, serosally added D-mannose and D-glucose increased $I_{sc}$ (Fig. 7), indicating that the basolateral side of ruminal epithelium is permeable by the hexoses, which are transported by GLUT 2 (5, 13). Although the underlying mechanisms of electronegeneity have not been clearly identified in the present study, they are probably not caused by direct charge transfer by the transport protein, because GLUT proteins are electroneutral carriers transporting sugars only (5, 15). Therefore, indirect effects of D-glucose or D-mannose on $I_{sc}$ must be assumed to result from intracellular metabolism of the substrates. Action of D-glucose metabolism and/or metabolites on $I_{sc}$ is further suggested by the observation that nonmetabolizable 3-OMG could not enhance $I_{sc}$ when added to the serosal side (Fig. 5). As for the nature of the metabolic effects of D-glucose and D-mannose, it may be due to an energy deficit of the ruminal preparation in the absence of metabolizable hexoses. The addition of hexoses could then have increased the ATP availability to the Na$^{+}$-K$^{+}$-ATPase, leading to a stimulation of electrogenic Na$^{+}$ transport caused by an elevated electrochemical driving force.

It is obvious that the indirect metabolic effects of D-glucose do not depend on the route of entry into the cell. Therefore, the electronegenic effects observed after serosal application of D-glucose are also involved in the effects of mucosally added D-glucose. As shown in Fig. 4, mucosal addition of D-glucose at a saturating concentration (10 mM) led to a greater increase of $I_{sc}$ than an equimolar addition of 3-OMG when the epithelia were preincubated under hexose-free conditions. On the other hand, mucosal addition of 10 mM D-glucose or 10 mM 3-OMG induced similar increases of $I_{sc}$ when metabolism was saturated by preincubation with 10 mM mannose (Fig. 8). These observations show that the electrogentic effect of apical D-glucose involves both a direct (SGLT dependent) and an indirect (metabolism dependent) component.

The identity of the electronegenic and Na$^{+}$-coupled uptake of mucosal D-glucose was further investigated at the molecular level. To date, three different Na$^{+}$-glucose cotransporter isoforms have been reported: one high-affinity Na$^{+}$-glucose cotransporter (SGLT-1) and two low-affinity Na$^{+}$-glucose cotransporters (SGLT-2 and SGLT-3; the latter is also named SAAT-pSGLT2; Ref. 11). Of these, SGLT-1 is the main isoform that mediates Na$^{+}$-coupled transport of D-glucose in the intestine of many species, including sheep (5, 11, 22, 28). Therefore, the present study investigated the possibility of SGLT-1 expression in the ruminal epithelium of sheep. By using specific primers for ovine intestinal SGLT-1 (GenBank X82410), an amplicon of expected length (435 bp) was recovered after RT-PCR from total mRNA isolated from ruminal mucosa. After TA cloning, restriction analysis of this cDNA and, finally, sequencing revealed that the RT-PCR amplicon truly originated from mRNA coding for ovine SGLT-1 (Fig. 9).

There is strong evidence that the electrophysiological and transport data obtained in this investigation result from translation of SGLT-1 mRNA into a functional protein. SGLT-2 has only a low affinity for D-glucose and a negligible capacity for 3-OMG transport and has only been demonstrated in the kidney (11, 18). Although the kinetic data of 3-OMG and D-glucose transport determined in the present study must be judged with care because they were determined either under steady-state conditions (Fig. 2) or indirectly by measuring the D-glucose- or 3-OMG-induced $I_{sc}$ (Fig. 8), they reveal that the affinity of the ruminal transporter to D-glucose and 3-OMG is rather high and resembles the one described in the brush-border membrane vesicles of the ovine intestine (22, 26). Furthermore, the $K_{m}$ of SGLT-1 for sodium is approximately ten times lower than the $K_{0.5}$ of SGLT-2. As a result,
[Na\(^+\)] becomes rate-limiting for SGLT-1 at \(\sim 10 \text{ mM}\), whereas [Na\(^+\)] restricts SGLT-2 function at \(\sim 100 \text{ mM}\) (11, 12). The lack of effect of decreasing the Na\(^+\) concentration from 140 mM to 40 mM (Fig. 3) is therefore indicative of a transporter with a high affinity for sodium, i.e., SGLT-1. SGLT-3, the third known member of the SGLT family, is also expressed in the intestine and has a high affinity for Na\(^+\) (11), but like SGLT-2, its affinity for d-glucose and glucose derivatives is low, a finding that does not agree with the kinetic parameters of d-glucose or 3-OMG transport determined in the ruminal epithelium (Figs. 2 and 8).

Although the present study clearly demonstrates the presence of SGLT-1 on both the mRNA and the functional level, the physiological role of ruminal SGLT-1 cannot be deduced directly. To postulate quantitatively important glucose absorption from the rumen would be in sharp contrast to previous experiments. Various in vivo and in vitro studies strongly indicate that glucose uptake into ruminal microorganisms and its subsequent catabolism to SCFA is the major route of glucose elimination from the ruminal fluid (4, 25), at least under physiological feeding conditions. Nevertheless, our study shows that microbial metabolism is certainly not the only way of ruminal glucose elimination. The ability of the ruminal epithelium to absorb d-glucose may even become significant if animals are changed to an energy-rich diet. Under these feeding conditions, the fermentative capacity of the microorganisms may be overtaxed and d-glucose may accumulate within the rumen (3, 8). It has repeatedly been demonstrated, however, that especially a sudden increase in easily fermentable carbohydrates induces intraruminal glucose accumulation and dysfermentation (i.e., ruminal lactic acidosis), whereas adapting ruminants to high-energy diets over several weeks triggers an intensive catabolism to SCFA is the major route of glucose elimination from the ruminal fluid (4, 25), at least under physiological feeding conditions. Nevertheless, our study shows that microbial metabolism is certainly not the only way of ruminal glucose elimination. The ability of the ruminal epithelium to absorb d-glucose may even become significant if animals are changed to an energy-rich diet. Under these feeding conditions, the fermentative capacity of the microorganisms may be overtaxed and d-glucose may accumulate within the rumen (3, 8). It has repeatedly been demonstrated, however, that especially a sudden increase in easily fermentable carbohydrates induces intraruminal glucose accumulation and dysfermentation (i.e., ruminal lactic acidosis), whereas adapting ruminants to high-energy diets over several weeks triggers an intensive catabolism to SCFA (4, 25), at least under physiological feeding conditions. Nevertheless, our study shows that microbial metabolism is certainly not the only way of ruminal glucose elimination. The ability of the ruminal epithelium to absorb d-glucose may even become significant if animals are changed to an energy-rich diet. Under these feeding conditions, the fermentative capacity of the microorganisms may be overtaxed and d-glucose may accumulate within the rumen (3, 8). It has repeatedly been demonstrated, however, that especially a sudden increase in easily fermentable carbohydrates induces intraruminal glucose accumulation and dysfermentation (i.e., ruminal lactic acidosis), whereas adapting ruminants to high-energy diets over several weeks triggers an intensive catabolism to SCFA is the major route of glucose elimination from the ruminal fluid (4, 25), at least under physiological feeding conditions. Nevertheless, our study shows that microbial metabolism is certainly not the only way of ruminal glucose elimination. The ability of the ruminal epithelium to absorb d-glucose may even become significant if animals are changed to an energy-rich diet. Under these feeding conditions, the fermentative capacity of the microorganisms may be overtaxed and d-glucose may accumulate within the rumen (3, 8). It has repeatedly been demonstrated, however, that especially a sudden increase in easily fermentable carbohydrates induces intraruminal glucose accumulation and dysfermentation (i.e., ruminal lactic acidosis), whereas adapting ruminants to high-energy diets over several weeks triggers an intensive catabolism to SCFA (4, 25), at least under physiological feeding conditions. Nevertheless, our study shows that microbial metabolism is certainly not the only way of ruminal glucose elimination. The ability of the ruminal epithelium to absorb d-glucose may even become significant if animals are changed to an energy-rich diet. Under these feeding conditions, the fermentative capacity of the microorganisms may be overtaxed and d-glucose may accumulate within the rumen (3, 8). It has repeatedly been demonstrated, however, that especially a sudden increase in easily fermentable carbohydrates induces intraruminal glucose accumulation and dysfermentation (i.e., ruminal lactic acidosis), whereas adapting ruminants to high-energy diets over several weeks triggers an intensive catabolism to SCFA as regulators of Na\(^+\) and Cl\(^-\) absorption in isolated sheep rumen mucosa. J Comp Physiol (B) 161: 419–426, 1991.

Glucose transport by SGLT-1 in ruminal epithelium normally fermentative form of intraruminal digestion (i.e., effective catabolism of d-glucose to SCFA by the intraruminal microorganisms; Refs. 4, 25), the ruminal epithelium still maintains the capacity for glucose absorption. The question of whether this capacity is an ontogenic remnant from the early monogastric phase in the development of the animal or whether it still plays an important role in the adult animal and its adaptation to high-energy diets remains to be clarified in further studies.

REFERENCES


