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Apical sodium–glucose co-transport can be regulated by blood-borne glucose in the ruminal epithelium of sheep (*Ovis aries*, Merino breed)

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The intestinal Na-dependent D-glucose co-transporter (SGLT)-1 in sheep is under dietary regulation by luminal substrates. The aim of the present study was to find out whether the SGLT-1 in the forestomach of sheep is also regulated by sugars. Furthermore, the location of a possible glucosensor (luminal *v.* intracellular *v.* basolateral) was to be elucidated. Ruminal epithelia of sheep (*Ovis aries*, Merino breed) were pre-incubated in Ussing chambers with various substrates on the mucosal (i.e. luminal) or serosal (i.e. blood) side. This pre-incubation period was followed by a second pre-incubation period without the tested substrates (washout period). Thereafter, apical D-glucose uptake by ruminal epithelial cells was determined with 200 μmol D-[^{14}C]glucose/l in the absence or co-presence of the SGLT-1 inhibitor, phlorizin. Pre-incubation with D-glucose on the mucosal side had no significant effect on apical D-glucose uptake ($P>0{\cdot}05$). In contrast, pre-incubation with D-glucose, D-mannose, 3-*O*-methyl-D-glucose or sucrose on the serosal side significantly increased D-glucose uptake compared with mannitol-treated controls ($P<0{\cdot}05$). Serosal pre-incubation with cellobiose or D-xylose had no effect. The stimulation of D-glucose uptake by serosal D-glucose pre-incubation was concentration dependent, with maximal stimulation at about 10 mmol/l. We conclude that the ruminal SGLT-1 can be up-regulated in a concentration-dependent manner by blood-borne D-glucose via an extracellular sugar-sensing mechanism.

Sodium–glucose-linked transport: Glucosensor: Rumen: Sheep

Adult ruminant animals achieve their energy demand largely by the absorption of SCFA from their forestomachs, especially from the rumen (Gäbel *et al.* 2002). The SCFA, in turn, are derived from fermentation of carbohydrates by the microbes in the rumen. Given the extensive carbohydrate fermentation, absorption of glucose has long been thought to be of no significance to the ruminant animal (Merchen, 1988; Britton & Krehbiel, 1993). However, recent studies have clearly demonstrated on molecular and functional levels that glucose can be taken up from the rumen of sheep and fallow deer (*Cervus dama*) via the sodium–glucose-linked transporter (SGLT)-1 (Aschenbach *et al.* 2000*a*,*b*, 2002*b*). SGLT-1, in turn, has been known for a long time to be the key absorptive mechanism for D-glucose in the intestine (Wright, 1993; Wood & Trayhurn, 2003).

The ruminal SGLT-1 of sheep is sensitive to hormonal regulation by adrenaline (Aschenbach *et al.* 2002*a*) similarly to the intestinal SGLT-1 of rats (Ishikawa *et al.* 1997). However, very little is known about a possible substrate regulation of SGLT-1 in ruminal epithelial cells. Substrate regulation of SGLT-1 has been well documented in the intestinal epithelium of sheep (Bauer *et al.* 1995; Shirazi-Beechey *et al.* 1995). The activity and expression of the intestinal SGLT-1 declines dramatically when the pre-ruminant lamb is weaned and matures, in parallel to a significant decrease in the levels of luminal monosaccharides entering the small intestine (Wood *et al.* 2000). The activity of the ovine intestinal SGLT-1 can be reconstituted by luminal, but not intravenous, infusion of glucose, suggesting direct dietary regulation (Shirazi-Beechey *et al.* 1994).

Possible substrate regulation of the ovine ruminal SGLT-1 was recently indicated by a decreased transport of the glucose analogue, 3-*O*-methyl-D-glucose, after 48 h food deprivation (Gäbel & Aschenbach, 2002). However, it remained unclear whether this was a specific effect attributable to the absence of carbohydrates. In addition, an up-regulation of SGLT-1 by energy-rich diets would be much more important for the productivity and well-being of the animal than down-regulation during starvation (Aschenbach *et al.* 2000*a*, 2002*a*). The present study was, therefore, intended to find out whether up-regulation of the ruminal SGLT-1 can be induced by D-glucose or other sugars and whether this occurs in a way similar to that observed in the small intestine of sheep.

Abbreviations: I_{sc}, short-circuit current; SGLT, sodium-dependent D-glucose co-transporter.

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Materials and methods

Animals and preparation of epithelia

Adult (2–5 years old) female sheep (*Ovis aries*, Merino breed) were fed good-quality meadow hay (first cut), and mineral blocks and water *ad libitum* for at least 14 d before the experiments. Sheep were stunned by bolt pistol and killed by exsanguination. The gastrointestinal tract was removed immediately from the abdominal cavity. Stripped ruminal epithelia were prepared from the ventral ruminal sac and mounted into Ussing chambers. Epithelial preparations were incubated with 15 ml buffer solution on their mucosal (luminal) and serosal (blood) sides under short-circuit conditions as described by Aschenbach *et al.* (2000*b*). Experiments were in accordance with the German legislation on the protection of animals and were communicated to the Regierungspräsidium Leipzig (AZ 74-9162.11-01-T53/01).

Buffer solutions

Buffer solutions used for washing, transport and incubation of epithelia contained (mmol/l): NaCl 75, KCl 5, $CaCl_2$ 1, $MgCl_2$ 1, NaH_2PO_4 1, Na_2HPO_4 2, L-glutamine 1, sodium acetate 10, sodium propionate 10, butyric acid 10, sodium-DL-lactate 5, HEPES (free acid) 10, mannitol 40, NaOH (283–293 mosmol/kg, pH 7·36–7·44) 10. Solutions were gassed with 100 % O_2 at 37°C.

Pre-incubation of epithelia with different substrates

Ruminal epithelia were pre-incubated with D-mannitol (control) or D-glucose, D-mannose, 3-*O*-methyl-D-glucose, sucrose, cellobiose or D-xylose at a concentration of 10 mmol/l on the mucosal or serosal side for 10 or 240 min. Some epithelia were pre-incubated with increasing concentrations of D-glucose (0·0–17·8 mmol/l) on the serosal side for 10 min. Transepithelial osmotic gradients and differences in osmolarity between treatments were avoided by compensatory mannitol additions. The pre-incubation period with substrates was followed by a second 10 min pre-incubation period without any of the substrates (washout period).

Glucose uptake technique

The glucose uptake technique for ruminal epithelia has been described in detail previously (Aschenbach *et al.* 2002*a*). Briefly, 200 μmol D-glucose/l spiked with 80·1 μCi D-[^{14}C]glucose/l were added to the mucosal side for 1 min. After three washings, the mucosal area previously exposed to the uptake buffer was covered with 4 ml ice-cold NaOH (100 mmol/l) for 3 min in a self-made lysing device. Cornified aggregates were removed from the harvested lysate by centrifugation (3000 ***g***, 15 min). The D-glucose and protein contents of lysates were determined in duplicate by scintillation counting and method of Smith *et al.* (1985) respectively.

Pairs of epithelia were incubated in the absence or the mucosal presence of the inhibitor of SGLT-1, phlorizin (100 μmol/l). The decrease in D-glucose uptake induced by phlorizin (i.e. phlorizin-sensitive D-glucose uptake) can be interpreted as the portion of D-glucose uptake attributable to SGLT-1. Phlorizin was dissolved in ethanol. Corresponding epithelia without phlorizin received an equivalent amount of ethanol.

Electrophysiological measurements

Experiments were conducted in the absence of electrochemical gradients, i.e. with buffer of the same composition on both sides of the epithelium and the transepithelial potential difference clamped to 0 mV (short-circuit conditions). Tissue conductance and short-circuit current (I_{sc}) were monitored continuously, as explained in detail by Aschenbach *et al.* (2000*b*). The I_{sc} is equivalent to the net charge transfer across the epithelium. To compare the effect of different substrates on I_{sc}, the increase in I_{sc} was calculated between the time points immediately before substrate additions and the time points immediately before washout (i.e. 10 or 240 min thereafter). The allotment of epithelia to different substrate treatments was based on their tissue conductance values about 30 min after mounting. Care was taken to have low- and high-conductance epithelia represented equally in all groups. Pairs of epithelial tissues used for determination of phlorizin-sensitive uptake were not allowed to differ in their conductance values by >25 %.

Statistical analyses

Arithmetic mean values are presented with their standard errors. Statistical differences were established by one-way or two-way repeated measurements ANOVA (normal distribution) or repeated measurements ANOVA on ranks (no normal distribution). Following ANOVA, groups that differed were isolated by the multiple comparison procedures of Student–Newman–Keul or Dunnett.

Chemicals

O_2 was supplied by Messer Griesheim (Krefeld, Germany). D-[U-^{14}C]Glucose was purchased from Amersham Pharmacia Biotech (Freiburg, Germany). The scintillation fluid, Aquasafe 300 Plus, was purchased from Zinsser Analytic (Maidenhead, Berks., UK). All other chemicals were supplied by either Merck (Darmstadt, Germany) or Sigma-Aldrich (Deisenhofen, Germany).

Results

Apical uptake of D-glucose was 56·1 (SEM 7·2) pmol/mg protein per min after control pre-incubation with D-mannitol on both sides. This basal uptake was reduced ($P<0·01$) to 36·0 (SEM 5·8) pmol/mg protein per min after inhibition of SGLT-1 by mucosal addition of phlorizin. Consequently, phlorizin-sensitive apical uptake of D-glucose was 20·1 (SEM 6·3) pmol/mg protein per min under control conditions. Pre-incubation of ruminal epithelia with D-glucose on the mucosal side did not result in a significant increase of phlorizin-sensitive D-glucose uptake (Expt 1 in Table 1). On the other hand, phlorizin-sensitive apical

uptake of D-glucose was more than doubled by pre-incubation with D-glucose on the serosal side ($P<0{\cdot}05$). A comparable increase in phlorizin-sensitive uptake of D-glucose was induced by serosal pre-incubation with mannose ($P<0{\cdot}05$; Expt 1 in Table 1).

The effectiveness of serosal D-glucose and D-mannose in increasing D-glucose uptake within only 20 min (i.e. 10 min pre-incubation + 10 min washout) indicated that genomic effects are most probably not involved. To substantiate this hypothesis, we tested whether an increase in pre-incubation time would further increase the stimulation of phlorizin-sensitive uptake by serosal D-glucose or D-mannose. However, phlorizin-sensitive apical uptake of D-glucose did not increase any further when increasing the pre-incubation time (with serosal D-glucose or D-mannose) from 10 to 240 min (Fig. 1(A)). Furthermore, a 240 min pre-incubation with D-glucose on the mucosal side was as ineffective in increasing D-glucose uptake as in a 10-min pre-incubation (Fig. 1(A)).

It was shown in a previous study that D-glucose application to ruminal epithelia induced an increase in I_{sc} that could be attributed partly (mucosal application) or completely (serosal application) to intracellular metabolism of D-glucose (Aschenbach *et al.* 2002*b*). Similarly, serosal mannose can be metabolized by the ruminal epithelial cells and thus increases I_{sc} (Aschenbach *et al.* 2000*b*). Therefore, the increase in I_{sc} within the period of substrate pre-incubation (ΔI_{sc}) was analysed to elucidate a possible link between hexose metabolism and the observed stimulation of phlorizin-sensitive D-glucose uptake. In comparison with control pre-incubations, the application of D-glucose or D-mannose (10 mmol/l) induced a moderate to marked ΔI_{sc} ($P<0{\cdot}05$; Expt 1 in Table 1; Fig. 1(B)). Serosal addition of D-glucose or D-mannose was more effective in increasing I_{sc} than mucosal addition of D-glucose ($P<0{\cdot}05$). The ΔI_{sc} values were not different with the 10 or 240 min pre-incubation protocol (Fig. 1(B)).

The effectiveness of serosal D-glucose in increasing apical D-glucose uptake and I_{sc} was dependent on the concentration of D-glucose during serosal pre-incubation. Both apical D-glucose uptake and ΔI_{sc} increased almost in parallel during 10 min pre-incubations with 0·0–8·9 mmol D-glucose/l on the serosal side ($P<0{\cdot}05$; FIg. 2).

The results described so far pointed to the possibility that serosally applied substrates could stimulate apical D-glucose uptake via the supply of metabolic energy. To test this hypothesis, ruminal epithelia were pre-incubated with the non-metabolisable D-glucose analogue, 3-*O*-methyl-D-glucose, two disaccharides (sucrose or cellobiose) and a pentose sugar (D-xylose) on the serosal side. None of these carbohydrates induced changes in I_{sc} at a concentration of 10 mmol/l (Table 2; Expts 5 and 6 in Table 1). After pre-incubation with non-metabolisable but cell-permeant 3-*O*-methyl-D-glucose, only total apical uptake of D-glucose was measured, which increased above mannitol-treated controls ($P<0{\cdot}05$; Table 2). Furthermore, an increase in phlorizin-sensitive uptake of D-glucose could be established for cell-impermeant sucrose, whereas cellobiose or D-xylose had no effect (Table 1).

Discussion

Several previous studies have suggested that the basolateral pole is the dominant site of D-glucose entry into ruminal epithelial cells in the living animal (Remond *et al.* 1993; Lozano *et al.* 2000). Following basolateral entry via a yet unidentified isoform of GLUT (Aschenbach *et al.* 2000*b*), D-glucose is metabolised to lactate or, to a much lesser extent, totally oxidised to CO_2 (Baldwin & McLeod,

Table 1. Effect of different 10 min pre-incubations with substrates on phlorizin-sensitive apical uptake of D-glucose and on the increase in short-circuit current (ΔI_{sc}) within 10 min after substrate applications in isolated ruminal epithelia of sheep*

(Mean values with their standard errors)

Substrate		Phlorizin-sensitive D-glucose uptake (pmol/mg protein per min)		ΔI_{sc} (μEq/cm^2 per h)	
Mucosal side	Serosal side	Mean	SEM	Mean	SEM
Expt 1 (*n* 9)					
Mannitol	Mannitol	20·1[a]	6·3	0·00[a]	0·01
D-Glucose	Mannitol	28·9[ab]	5·5	0·27[b]	0·09
Mannitol	D-Glucose	45·6[b]	6·4	0·97[d]	0·20
Mannitol	D-Mannose	43·9[b]	9·2	0·56[c]	0·11
Expt 5 (*n* 7)					
Mannitol	Mannitol	43·3[a]	12·8	0·01	0·01
Mannitol	Sucrose	60·5[b]	14·9	−0·01	0·01
Mannitol	Cellobiose	29·0[a]	9·9	−0·02	0·02
Expt 6 (*n* 6)					
Mannitol	Mannitol	40·0	14·6	0·01	0·01
Mannitol	D-Xylose	36·8	9·3	0·00	0·01

[a,b,c,d] For each experiment, mean values within a column with unlike superscript letters were significantly different ($P<0{\cdot}05$).

* The ruminal epithelia were pre-incubated either bilaterally with 10 mmol D-mannitol/l or on one side with the indicated substrate (10 mmol/l) for 10 min, followed by a further pre-incubation period in substrate-free buffer for 10 min before a 1 min glucose uptake period. Phlorizin-sensitive D-glucose uptake was calculated for each animal as the difference between the uptakes in the absence and presence of phlorizin (100 μmol/l). For details of procedures, see p. 778.

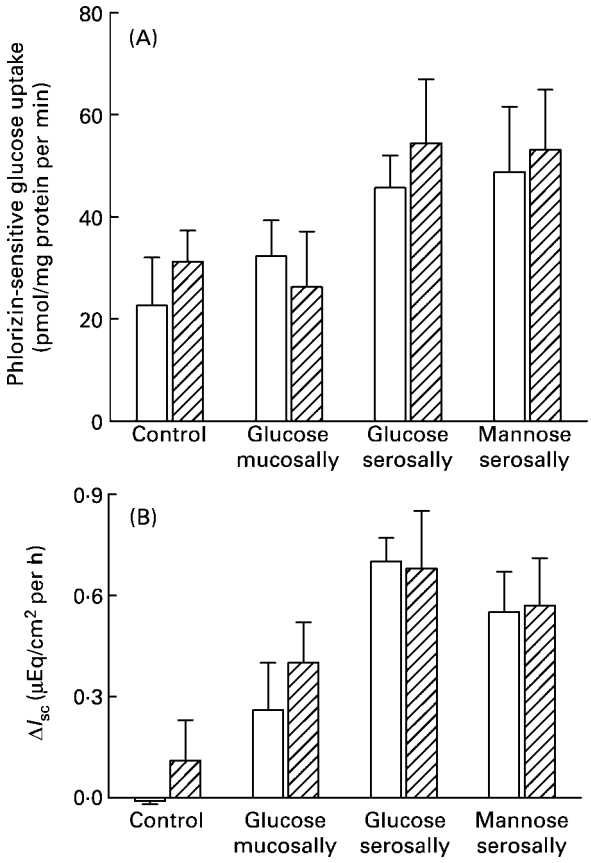

Fig. 1. Effect of pre-incubation with 10 mmol/l-D-glucose or 10 mM-D-mannose for 10 or 240 min on phlorizin-sensitive D-glucose uptake (A) and on increases in short-circuit current (I_{sc}) (B) during the 10 (□) or 240 (▨) min of substrate presence in isolated ruminal epithelia of sheep. For details of procedures, see p. 778. Uptake was measured at a concentration of 200 μmol/l-D-glucose after washing out the pre-incubation substrate for 10 min. On two-way repeated measurements ANOVA, D-glucose uptakes and I_{sc} were increased equally by serosal pre-incubations with D-glucose or D-mannose ($P<0·05$). Mucosal pre-incubation with D-glucose had no effect on D-glucose uptake but increased I_{sc} to values below those after serosal D-glucose or D-mannose pre-treatment ($P<0·05$). The time of substrate presence had no effect on glucose uptake or I_{sc}. Values are means with their standard errors shown by vertical bars (n 6).

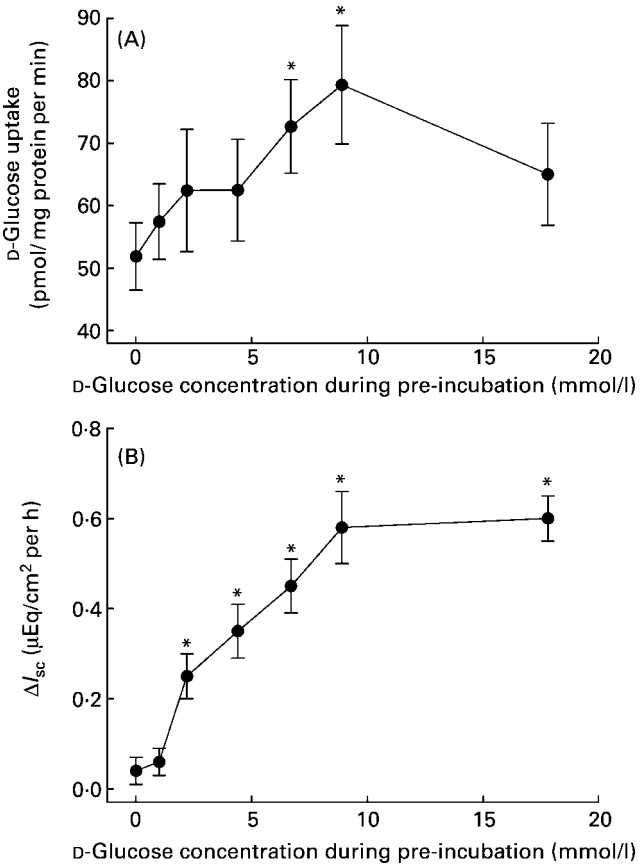

Fig. 2. Influence of D-glucose concentration on the serosal side (A) on apical D-glucose uptake and (B) on short-circuit current (I_{sc}) in isolated ruminal epithelia of sheep. For details of procedures, see p. 778. The ruminal epithelia were pre-incubated with increasing concentrations of D-glucose on the serosal side for 10 min, followed by a further pre-incubation period in D-glucose-free buffer for 10 min before a 1 min D-glucose uptake period. Values are means with their standard errors shown by vertical bars (n 9). Mean values were significantly different from the control values (0 mmol/l): *$P<0·05$.

2000). The basolateral uptake and metabolism of D-glucose can be visualised by an increase in I_{sc} in Ussing-chambered ruminal epithelia (Aschenbach *et al.* 2000*b*, 2002*b*). The relative contribution of D-glucose to the epithelial cell energy requirement, however, is less than that of the SCFA taken up from the lumen (Baldwin & McLeod, 2000). Thus, the net disappearance of D-glucose from blood into the ruminal wall is not very high. It amounts to only 6–12 mmol/d in adult sheep on a hay-based diet, which is approximately ten times less than mesenteric glucose disappearance (Remond *et al.* 1993; Han *et al.* 2002).

Table 2. Effect of a serosal 10 min pre-incubation with 3-*O*-methyl-α-D-glucose (3-OMG) on apical uptake of D-glucose and on the increase in short-circuit current (ΔI_{sc}) in isolated ruminal epithelia of sheep*

(Mean values with their standard errors)

Substrate		D-glucose uptake (pmol/mg protein per min)		ΔI_{sc} (μEq/cm^2 per h)	
Mucosal side	Serosal side	Mean	SEM	Mean	SEM
Expt 4 (n 7)					
Mannitol	Mannitol	55·8[a]	6·5	−0·01	0·01
Mannitol	3-*O*MG	85·9[b]	8·6	0·00	0·00

[a,b] Mean values within a column with unlike superscript letters were significantly different ($P<0·05$).

*The ruminal epithelia were pre-incubated with D-mannitol or 3-*O*-methyl-D-glucose (3-*O*MG) (10 mmol/l) on the serosal side for 10 min, followed by a further pre-incubation period in 3-OMG-free buffer for 10 min before a 1 min glucose uptake period. For details of procedures, see p. 778.

The extent to which the basolateral net flux of D-glucose is complemented or affected by an apical uptake from the ruminal content has not been measured directly. Intra-ruminal concentrations of glucose appear to be very low, because it is rapidly converted to SCFA and lactic acid by ruminal bacteria (Russell & Gahr, 2000). However, periods with appreciable glucose concentrations can be expected with diets containing either large amounts of glucose itself or large amounts of other very easily fermentable carbohydrates (Kajikawa *et al.* 1997). Such concentrate diets are being used increasingly to enhance the performance of ruminant animal production, especially milk yield in cattle (Gäbel *et al.* 2002). We have suggested that glucose absorption from the rumen occurs under these circumstances and is advantageous. Apart from some energetic benefits, it would contribute to decreased luminal glucose availability and thus decrease the risk of ruminal lactic acidosis, a disorder caused by excessive acid release from bacterial fermentation (Aschenbach *et al.* 2000*a*, 2002*a*).

Absorption of D-glucose from the rumen of sheep depends on its Na-dependent uptake across the apical cell membrane via SGLT-1 (Aschenbach *et al.* 2000*a*,*b*, 2002*a*). The capacity of the Na-dependent uptake was previously assessed in the antibiotic-treated reticulorumen of sheep *in vivo* (Aschenbach *et al.* 2000*a*). At a concentration of 0·50 mmol D-glucose/l, 0·07 mmol D-glucose was extracted from each litre of an intra-ruminal solution within 1 h (i.e. 13 %/h). Because the concentration of 0·50 mmol/l was well above the concentration of half-maximal D-glucose transport by SGLT-1 ($K_{0.5}$ 0·28; Aschenbach *et al.* 2000*b*), it was initially assumed that D-glucose absorption would not rise much further at higher D-glucose concentrations. However, in the same study, D-glucose disappearance rate from the reticulorumen amounted to 2·90 mmol/(l · h) at a concentration of 12·00 mmol/l (i.e. 24 %/h; Aschenbach *et al.* 2000*a*). This is quite a remarkable value compared with the net glucose disappearance from blood cited earlier (Remond *et al.* 1993; Han *et al.* 2002). On the other hand, the sharp increase in glucose absorption between luminal concentrations of 0·50 and 12·00 mmol/l can only be explained by some kind of substrate regulation of SGLT-1. Accordingly, ruminal SGLT-1 activity decreased in another study after 48 h feed deprivation (Gäbel & Aschenbach, 2002). The presence and characteristics of possible substrate regulation were addressed in the present study.

Our present results demonstrate that apical phlorizin-sensitive D-glucose uptake by ruminal epithelia is stimulated by D-glucose presence on the serosal, i.e. blood, side (Fig. 1(A)). This is in contrast to the intestine, where substrate regulation of SGLT-1 is mainly attributed to the availability of substrates in the intestinal lumen (Shirazi-Beechey *et al.* 1991; Shirazi-Beechey, 1996; Dyer *et al.* 1997). Nevertheless, the effects of sugars on SGLT-1 *per se* may bear similarities apart from the different location of glucosensors in the rumen *v.* intestine. Concerning the intestine, it is known that the luminal glucosensor has a broad substrate specificity, is located on the external face of cells and operates independently from D-glucose or other substrate metabolism (Dyer *et al.* 2003). All these characteristics apply also to the blood-side-directed glucosensor in the ruminal epithelium: the ruminal SGLT-1 can be stimulated by glucose itself as well as by other sugars. Among the stimulating sugars were those that are transported by apical SGLT-1 as well as basolateral GLUT (D-glucose, 3-*O*-methyl-glucose), those that are transported by GLUT but poorly or not by SGLT-1 (D-mannose) and those that are transported by neither GLUT nor SGLT-1 (sucrose). In this regard, the efficacy of sucrose in stimulating SGLT-1 also proves that the D-glucose sensor has an extracellular location because sucrose as a disaccharide is almost membrane impermeant and cannot accumulate inside the cells. It can be excluded that the effect of sucrose involved extracellular sucrose hydrolysis (to give D-glucose). Such indirect action of sucrose via D-glucose would have been recognisable by a rise in I_{sc}, which was absent. The rise in I_{sc} due to D-glucose can be attributed to its intracellular metabolism after basolateral uptake via GLUT (Aschenbach *et al.* 2000*b*, 2002*b*). A similar metabolic effect was seen with D-mannose but not 3-*O*-methyl-glucose or sucrose. Therefore, metabolism of substrates is not essential for the stimulation of the ruminal SGLT-1.

It has been proven for the intestine that the signalling pathway of the glucosensor involves generation of cAMP and activation of protein kinase A (Dyer *et al.* 2003). It is conceivable that the same pathway is used in the ruminal epithelium. We have shown previously that experimental stimulation of cAMP generation by forskolin can increase the activity of the ruminal SGLT-1 (Aschenbach *et al.* 2002*a*). However, both the increase in SGLT-1 activity induced by forskolin (Aschenbach *et al.* 2002*a*) and the substrate regulation of SGLT-1 (present study) occur rather rapidly in the ruminal epithelium (from s to min). The rapid onset of substrate-induced increases in SGLT-1 activity is in contrast to observations in the intestine and strongly suggests that the regulation in the ruminal epithelium is not transcriptional. With regulation of SGLT-1 at the mRNA or protein expression level, one would have expected a slower response or, at least, a fortification of the stimulatory effect when increasing the pre-incubation time with substrates towards several hours. The latter was not obvious (Fig. 1).

Increases in plasma glucose levels have previously been implicated in enhanced functional activity of the intestinal SGLT-1 during diabetes. However, the blood-side regulation of the ruminal SGLT-1 in the present study appears to be different from that described earlier in diabetic rats (Debnam, 1994). This effect in diabetic rats has been attributed to an increased Na^+ driving force due to a decrease in apical membrane Na^+ conductance (Debnam, 1994; Sharp *et al.* 1997). However, the increased net absorption of positive charges (I_{sc}) after serosal D-glucose application in the present study supports an increased electrogenic Na^+ transfer across the apical membrane rather than a decrease. In addition, blood-side regulation in the rat intestine seems more strictly bound to D-glucose and not to other substrates (Sharp & Debnam, 1994). An effect of diabetes on SGLT-1 has also been described in human subjects, namely an increase in the mRNA and protein expression of SGLT-1. However, the subjects of the human study had been on hypoglycaemic treatment,

which could indicate an influence of diabetes on SGLT-1 expression even in the absence of hyperglycaemia (Dyer *et al.* 2002). In contrast, a specific effect of basolateral D-glucose on SGLT-1 function was investigated and established in the present study. It turns out that substrate stimulation of the ruminal SGLT-1 by blood-borne D-glucose could present a new regulatory principle, which seems related to the regulation of the intestinal SGLT-1 by luminal substrates.

The blood-side stimulation of the ruminal SGLT-1 was dose-dependent in the range of potential plasma glucose concentrations (Fig. 2(A)), suggesting a physiological role in the living animal. The plasma glucose concentration of ruminant animals can be influenced by the diet. For example, sheep grazing on spring pasture have significantly greater plasma glucose concentrations than the same animals on autumn pasture (4·8 *v.* 3·6 mmol/l; Maas *et al.* 2001). These differences in blood glucose availability are mostly attributed to a changing supply of glucogenic substrates by ruminal bacteria (e.g. propionate) or to post-ruminal absorption of D-glucose (Merchen, 1988; Bauer *et al.* 1995; Russell & Gahr, 2000). However, the different content of soluble carbohydrates in spring *v*. autumn grass (138 *v.* 77 g/kg DM) presents an equally plausible explanation. Direct ruminal infusions of moderate amounts of D-glucose (about 3–4 g/kg body weight) increase plasma glucose concentration of sheep from about 3 to 6 mmol/l if given as a bolus (Dougherty *et al.* 1956) or from 3·1 to 4·1 mmol/l if infused over 2 h (Giduck *et al.* 1988). The immediate increase after a bolus application (Dougherty *et al.* 1956) points to a significant contribution of forestomach glucose absorption to blood glucose availability under these conditions. However, with starch as a concentrate energy source, changes in plasma glucose concentrations are usually much more subtle (Swanson *et al.* 2000), as long as ruminal acidosis is not induced. Thus, different carbohydrates seem to contribute differently to plasma glucose availability. Non-nutritional factors that influence plasma glucose levels are animal species (e.g. deer > sheep; Webster *et al.* 1996; El-Sherif & Assad, 2001), breed (lean > fat genotype sheep; Francis *et al.* 1999) and reproductive stage (lactators > non-lactators; El-Sherif & Assad, 2001; Gow *et al.* 2003).

From a quantitative point of view, the blood-side-directed glucosensor discovered in the present study explains only part of the stimulation observed previously *in vivo* (Aschenbach *et al.* 2000*a*). This suggests that it may be either amplified or complemented by other regulatory mechanisms *in vivo*. Some plausible suggestions for these additional mechanisms are hormonal and/or neuronal stimuli, or changes in epithelial blood supply. These possibilities will have to be addressed in future studies.

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