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Influence of abomasal carbohydrates on small intestinal sodium-dependent glucose cotransporter activity and abundance in steers


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ABSTRACT: Most animals adapt readily to increased supplies of carbohydrate in the intestinal lumen by increasing enzymes for degradation and increasing glucose transporter activity. However, the extent of upregulation of Na⁺-dependent glucose cotransporter 1 (SGLT1) activity and content in response to increased delivery of carbohydrate to the small intestinal lumen of ruminants is unclear. Therefore, an experiment was conducted to determine the effect of glucose and starch hydrolysate on the activity and abundance of SGLT1 in the small intestine of steers. In a randomized complete block design, 40 crossbred beef steers (243 ± 2 kg BW) were fed 0.163 Mcal of ME/(kg BW0.75/d; W), 0.215 Mcal of ME/(kg BW0.75/d; 2M), or 0.163 Mcal ME/(kg BW0.75/d) and infused for 35 d into the rumen (R) or abomasum (A) with 12.6 g/(kg BW0.75/d) of starch hydrolysate (S) or into the abomasum with 14.4 g/(kg BW0.75/d) of glucose (G). Steers were slaughtered, and brush-border membrane vesicles were prepared from the small intestinal samples obtained from five equidistant sites along the intestine. Maltase activity in vesicles and homogenates differed with intestinal sampling site (quadratic, \( P < 0.001 \)). Steers on the AG treatment yielded a greater intestinal maltase activity (38 nmol glucose·mg protein⁻¹·min⁻¹) compared with the AS, RS, W, or 2M treatments (34, 26, 23, and 23 nmol glucose·mg protein⁻¹·min⁻¹, respectively [SEM = 3; \( P = 0.02 \)]). Sodium-dependent glucose uptake averaged 18.4 ± 3.94 pmol glucose/(mg protein⁻¹·min⁻¹) and was not affected by treatment, but uptake decreased distally along the intestine (\( P < 0.001 \)). There was no effect of treatment on SGLT1 protein abundance, but SGLT1 protein abundance increased linearly from the duodenum to the ileum (\( P = 0.05 \)). The inverse relationship between glucose uptake and SGLT1 abundance suggests that the regulation of brush border Na⁺-dependent glucose transport capacity is complex, involving factors other than the presence of luminal carbohydrate.

Key Words: Cattle, Glucose, Maltase, Sodium-Dependent Glucose Cotransporter, Starch

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Introduction

The ruminant small intestine has evolved to handle a small starch supply, presumably a consequence of low starch intake and high ruminal carbohydrate fermentation. However, modern production feeding systems promote the consumption of high-starch diets, and therefore more starch escapes ruminal fermentation and reaches the small intestine. Finishing beef cattle consuming high-concentrate diets can have upwards of 1 kg of starch flowing to the small intestine daily (Kreikemeier et al., 1991). Following starch hydrolysis, active glucose transport is the major mechanism of glucose absorption from the small intestine, and thus, it has been suggested that active Na⁺-dependent glucose transport by Na⁺-dependent glucose cotransporter 1 (SGLT1) could be a limit to starch assimilation in the small intestine of ruminants (Kreikemeier et al., 1991; Bauer et al., 2001a). Adult ruminants consuming a forage-based diet typically have very low SGLT1 activity (Wood et al., 2000), but there is evidence that SGLT1 can be upregulated in response to luminal substrate (Shirazi-Beechey et al., 1991; Bauer et al., 2001b). However, there are conflicting data concerning the extent of upregulation of SGLT1-mediated glucose absorption in response to luminal carbohydrate (Bauer et al., 2001a,b). Adaptive responses have been greater in sheep (Shirazi-Beechey et al., 1991; Bauer et al., 2001b)
and there is a scarcity of information on the response of SGLT1 activity and abundance to abomasal glucose infusion in cattle. The objectives of the current study were to 1) determine the influence of abomasal glucose on Na+-dependent glucose uptake in cattle, and 2) determine whether a relationship exists between Na+-dependent glucose uptake and SGLT1 abundance in cattle.

Materials and Methods

Steers and Treatments

Forty crossbred beef steers (243 ± 2 kg BW) were fitted with abomasal cannula according to the procedure of Driedger et al. (1970) with the following modifications. The cannulas were constructed of medical-grade Tygon tubing (64 mm i.d., 80 mm o.d.) that had a 1.3-cm Tygon cuff and a Teflon retaining washer (3.8-cm diameter) positioned 3.8 cm from the tip of the cannula. Ten days after insertion of the abomasal cannula, the steers were fitted with a similar cannula in the rumen. All procedures were approved by the Beltsville Animal Care and Use Committee. Steers were housed in individual tie stalls (12.2 × 17.1 m) in a temperature-controlled barn and were exercised for 1 h twice weekly. The steers received a 19.5% CP (DM basis) pelleted diet (89.45% orchardgrass hay, 5.0% corn gluten meal, 5.0% Soypass [Ligotech U.S.A., Rothschild, WI], 0.50% trace mineral salt [92 to 98.5% NaCl, 0.35% Zn, 0.34% Fe, 0.20% Mn, 330 ppm Cu, 70 ppm I, 50 ppm Co, and 90 ppm Se], and 0.05 % vitamin premix [4,400 IU of vitamin A, 880 IU of vitamin D, and 0.6 IU of vitamin E/kg of diet]; DM basis) in 12 portions daily at 2-h intervals and were allowed ad libitum access to water. Metabolizable energy values for the diets were calculated based on NRC (1996) values, and the energy values for the starch and glucose infusates were based on their respective heats of combustion. In a randomized complete block design, the steers were divided into eight blocks of five animals each, and treatments were assigned randomly within block (Table 1). To achieve isoenergetic infusions, the amount of starch hydrolysate or glucose, 12.6 and 14.4 g/(kg BW0.75·d), respectively, was suspended in tap water to a final volume of 5 L and administered daily over a 22-h period at a rate of 3.8 mL/min. The infusion period began 14 d after the insertion of the cannula, and steers were adapted to dietary intake and carbohydrate infusion over the first 6 d of the 35-d infusion period. The starting dates of the infusion were staggered both between and within blocks, whereby steers were slaughtered, one or two per day, at the end of the 35-d infusion period.

Tissue Collection

Steers were stunned by captive bolt, exsanguinated, and eviscerated. After slaughter, the small intestine was obtained, trimmed of associated fat, and looped around pegs placed 2 m apart on a wet, laminated board. Five 1-m pieces from equidistant sites along the intestine were retrieved (duodenum, jejunum 1, jejunum 2, jejunum 3, and ileum), with the duodenal section being immediately distal to the pylorus and the ileal section being immediately proximal to the ileal-cecal junction. The sections were rinsed with ice-cold saline solution to remove chyme and divided into four equal pieces. Each piece was everted with sponge forceps and rinsed three times by emersion in ice-cold saline. The rinsed pieces were each placed in a whirl-pack bag, snap-frozen in liquid N, and stored at −80°C until vesicle preparation. The remaining small intestine, minus the excised pieces, was rinsed with tap water, dripped dry, and weighed.

Brush Border Membrane Vesicle Preparation

Brush-border membrane vesicles were prepared from the frozen intestinal tissue according to the procedure of Kessler et al. (1978), as modified by Shirazi-Beechey et al. (1991) and Bauer et al. (2001b). The entire procedure was performed either on ice or at 4°C. Briefly, the frozen tissue pieces were weighed and thawed in buffer (100 mM mannitol, 2 mM HEPES/Tris buffer, pH 7.1) in a chilled beaker. Once thawed, the tissue was cut into 1-cm pieces with scissors and Russian thumb forceps in a chilled Petri dish. The tissue was returned to the beaker, and the tissue suspension was subjected to vibration (setting No. 80) for two 1-min intervals (Chemap AG CH-8604, Volketswil, Switzerland). The suspension was then filtered through a chilled Buchner

### Table 1. Treatment structure and designations for determining the effect of substrate on glucose transport in steers

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Designation</th>
<th>Intake, Mcal of ME/(kg BW0.75·d)</th>
<th>Infusion, g/(kg BW0.75·d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>W</td>
<td>0.163</td>
<td>0</td>
</tr>
<tr>
<td>High intake</td>
<td>2M</td>
<td>0.215</td>
<td>0</td>
</tr>
<tr>
<td>Ruminal starch</td>
<td>RS</td>
<td>0.163</td>
<td>12.6</td>
</tr>
<tr>
<td>Abomasal starch</td>
<td>AS</td>
<td>0.163</td>
<td>0</td>
</tr>
<tr>
<td>Abomasal glucose</td>
<td>AG</td>
<td>0.163</td>
<td>0</td>
</tr>
</tbody>
</table>

*All values of 0 represent a 5 L/d water infusion, with no starch or glucose.

*Glucose infusion represents an equivalent quantity of glucose infused as starch infusions.
funnel into a chilled 250-mL graduated cylinder. The volume of the filtrate was recorded, and the filtrate was returned to the 400-mL beaker. The beaker of filtrate was placed in an ice bath and stirred. Duplicate samples (2 mL) were obtained for further analysis, and these samples were considered to be the homogenate fraction. Magnesium chloride of a known concentration, determined via atomic absorption spectroscopy (ATI Unicam 99, Cambridge, U.K.), was added to the suspension in order to achieve a 10 mM concentration, and the solution was stirred gently for 20 min. After 20 min of gentle stirring, the cell suspension was subjected to differential centrifugation (5 min at 3,000 × g, 30 min at 30,000 × g, Sorvall RC5B Plus, Newtown, CT). The resulting pellet was resuspended, using a 23-gauge needle, in buffer (100 mM mannitol, 20 mM HEPES/Tris, pH 7.5), and the resuspended pellet was homogenized by 10 strokes in a Potter-Elvehjem (Teflon/glass) tissue grinder and centrifuged (30 min at 30,000 × g). The final pellet was resuspended with a 27-gauge needle 10 times, with special care so as not to create air bubbles. The vesicles were immediately aliquoted, placed in cryovials, and stored in liquid N.

Assays

Protein concentration of the homogenates and vesicles was assessed (Lowry et al., 1951) using BSA as the protein standard. Maltase was a marker enzyme for enrichment of the brush border membrane (Galand, 1989), and the method of Turner and Moran (1982) was used to measure maltase activity, with 25 mM phosphate (pH 6.3) buffer and 30 mM maltose. Labeled glucose (Infinity Glucose Reagent; Sigma Diagnostics, St. Louis, MO) was measured using COBAS Fara II (Roche, Montclair, NJ). Maltase activity was expressed as μmol of product/(min·mg of protein). Enrichment of maltase activity in the vesicles was determined to be the quotient of vesicle maltase activity and homogenate maltase activity.

Glucose uptake was measured at 37°C by placing 5 μL of vesicles (50 μg of protein) into prewarmed microcentrifuge tubes. Prewarmed incubation media containing either 100 mM sodium or potassium thiocyanate, 99.8 mM mannitol, 20 mM HEPES/Tris (pH 7.5), and 200 μM glucose (1.0 μCi [U-14C]-d-glucose) were added to the vesicles. Glucose uptake was terminated at 3 s by addition of 1.0 mL of ice-cold stop solution (150 mM NaCl and 250 μM phenol red; Bauer et al., 2001a,b). An aliquot (0.9 mL) was removed and rapidly filtered through a 0.45-μm cellulose Meritcell membrane (Pall Corp., Ann Arbor, MI). The entire membrane was thoroughly rinsed with stop solution (5 × 1 mL). The membrane was placed in a 20-mL scintillation vial, and the vial was filled with 12 mL of scintillation cocktail (Scintisafe Plus 50% advanced Safety LSC Cocktail, Fisher Scientific, Fair Lawn, NJ). Samples were counted for radioactivity using the Quantasam program (TriCarb 2900TR Liquid Scintillation Analyzer, Meridan, CT). Glucose uptake measurements were repeated five times in the presence of Na+ and K+.

Sodium-dependent glucose uptake was calculated by subtracting the uptake value obtained with the potassium thiocyanate incubation from that obtained with the sodium thiocyanate incubation.

Immunoblots

Vesicle and homogenate samples (40 μg of protein/lane) were incubated at 60°C and separated by 7.5% SDS PAGE (Laemmli, 1970). The proteins were electro-transferred to 0.45-μm nitrocellulose membranes and visualized by Fast-green (Fisher Scientific, Pittsburgh, PA) staining of the membrane. To indicate brush border membrane purity, membranes were probed serially for SGLT1, glucose transporter type 2 (GLUT2), and alkaline phosphatase. Membranes were blocked for 1.5 h in blocking solution (2.3% Carnation nonfat dry milk in 30 mM Tris base, 300 mM NaCl, 0.1% (vol/vol) Tween 20, pH 7.5). Membranes were placed in a miniblottter (Miniblotter 28, Immunetics, Cambridge, MA), and each sample lane was loaded with 50 μL of an anti-SGLT1 antibody (rabbit anti-rabbit SGLT1; 1:325 dilution; 100 μg/μL initial concentration; Alpha Diagnostic, San Antonio, Texas) in blocking solution and hybridized for 1 h at room temperature on a rocking platform. Membranes were rinsed in the miniblottter with 150 mL of blocking solution each, removed from the miniblottter, and rinsed for an additional 5 min with blocking solution. Horseradish peroxidase-conjugated secondary antibody (donkey anti-rabbit immunoglobulin; 1:5,000 dilution; Amersham Piscataway, NJ) was applied to each membrane for 1 h. The membranes were rinsed five times for 5 min with blocking solution and one time for 10 min in TBS/Tween (30 mM Tris base, 300 mM NaCl, 0.1% [vol/vol] Tween 20, pH 7.5). Membranes were gently blotted to remove excess moisture, and chemiluminescent substrate (Pierce Supersignal, Rockford, IL) was applied for 5 min. Membranes were gently blotted, wrapped in plastic wrap (Saran Classic, Racine, WI), exposed to film (Amersham Hyperfilm, Buchinghamshire, U.K.), and developed (Kodak M35A XLPlus, Cleveland, OH). In preparation for the next probe, the membranes were stripped (69 mM Na2CO3, 7.8% sodium or potassium thiocyanate incubation from that obtained with the sodium thiocyanate incubation. The membranes were hybridized in the miniblottter with 150 mL of blocking solution each, removed from the miniblottter, and rinsed for an additional 5 min with blocking solution. Horseradish peroxidase-conjugated secondary antibody (donkey anti-rabbit immunoglobulin; 1:5,000 dilution; Amersham Piscataway, NJ) was applied to each membrane for 1 h. The membranes were rinsed five times for 5 min with blocking solution and one time for 10 min in TBS/Tween (30 mM Tris base, 300 mM NaCl, 0.1% [vol/vol] Tween 20, pH 7.5). Membranes were gently blotted to remove excess moisture, and chemiluminescent substrate (Pierce Supersignal, Rockford, IL) was applied for 5 min. Membranes were gently blotted, wrapped in plastic wrap (Saran Classic, Racine, WI), exposed to film (Amersham Hyperfilm, Buchinghamshire, U.K.), and developed (Kodak M35A XLPplus, Cleveland, OH). In preparation for the next probe, the membranes were stripped (69 mM SDS, 62 mM Tris Base, 7.8 μL/mL β-mercaptoethanol) in a 60°C water bath for 30 min. Probing for GLUT 2 involved an initial 2-h block in blocking solution (2.5% Carnation nonfat dry milk in 30 mM Tris base, 150 mM NaCl, pH 7.5). Membranes were hybridized in the miniblottter with 50 μL of anti-GLUT2 antibody per sample lane for 1.5 h (rabbit anti-rat GLUT2; 1:165 dilution; 100 μg/μL initial concentration; Alpha Diagnostic). After hybridization, each membrane was rinsed in the miniblottter with 240 mL of blocking solution. Upon removal from the
miniblotters, membranes were rinsed three times for 5 min in blocking solution. Horseradish peroxidase-conjugated secondary antibody (donkey anti-rabbit immunoglobulin; 1:5,000 dilution) was applied to each membrane for 1.2 h. The remaining rinses and chemiluminescent visualization part of the procedure was the same as that followed for the SGLT1 probe. After visualization, the membranes were stripped again.

Probing for alkaline phosphatase involved an initial 1.5-h incubation in blocking solution (2.0% nonfat dry milk in 10 mM Tris Base, 200 mM NaCl, pH 7.5). Membranes were hybridized with antialkaline phosphatase antibody (calf anti-rabbit alkaline phosphatase; 1:10,000; 10 mg/mL initial concentration; Biodesign, Saco, ME) for 1 h in individual tubes on a turning platform. After hybridization, the membranes were rinsed five times for 5 min in blocking solution. Horseradish peroxidase-conjugated secondary antibody (donkey anti-rabbit immunoglobulin; 1:5000 dilution) was applied to each membrane for 1 h. The remaining rinses, as well as the chemiluminescent visualization part of the procedure were the same as that followed for the SGLT1 probe.

Determination of Relative Protein Abundance and Molecular Size

Digital images of the autoradiographic film from the immunoblots were scanned (HP DeskScan II, Hewlett-Packard, Palo Alto, CA), and scanning densitometry was performed and evaluated as described by Swanson et al. (2000) with the UN-SCANIT software program (Silk Scientific Corp., Orem, UT). Densitometric values were reported as arbitrary units, and an average background value for each autoradiograph was subtracted from the signal values on that respective autoradiograph. Enrichment values for SGLT1, GLUT2, and alkaline phosphatase were obtained by dividing the vesicle signal value by the homogenate signal value for each intestinal section. The densitometric values obtained for assessing SGLT1 abundance throughout the small intestine were normalized to the duodenal sample on each respective blot by dividing the densitometric value for each intestinal section by that for the duodenum. Apparent migration weights of SGLT1, GLUT2, and alkaline phosphatase were calculated by regressing the distance traveled against the migration weights of known markers, ranging from 184.5 to 9.1 kDa (Benchmark Prestained Protein Ladder, Invitrogen Corp., Carlsbad, CA).

Statistical Analyses

Vesicle and homogenate maltase activities, maltase enrichments, SGLT1 abundance, and glucose uptake data were analyzed as a split-plot design using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC). The whole-plot model was block and treatment, and the whole-plot error term was block \( \times \) treatment. The split-plot model included intestinal section and treatment \( \times \) intestinal section using residual sums of squares as an error term. Contrasts for linear and quadratic effects of intestinal sampling site were constructed. Intestinal lengths and weights were analyzed using a model that included block and treatment with the residual used as the error term. For all data, differences were considered to be significant when \( P < 0.05 \). Treatment differences were separated by the LSD procedure of SAS protected by a significant \( F \)-test.

Table 2. Enrichments of Na\(^{+}\)-dependent glucose cotransporter 1 (SGLT1), glucose transporter 2 (GLUT2), and alkaline phosphatase in brush border membrane vesicles from steers infused with differing carbohydrates

<table>
<thead>
<tr>
<th>Protein</th>
<th>Average enrichment(^a)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGLT1(^b)</td>
<td>9.22</td>
<td>1.94</td>
</tr>
<tr>
<td>GLUT2(^b)</td>
<td>1.01</td>
<td>0.15</td>
</tr>
<tr>
<td>Alkaline phosphatase(^c)</td>
<td>6.64</td>
<td>2.05</td>
</tr>
</tbody>
</table>

\(^a\)Enrichment = vesicle densitometric value/homogenate densitometric value

\(^b\)\(n = 23\) intestinal sections.

\(^c\)\(n = 12\) intestinal sections.

Results

Effects of control (W), high-energy intake (2M), ruminal starch infusion (RS), abomasal starch infusion (AS), or abomasal glucose infusion (AG) on changes in maltase activity, enrichment, and Na\(^{+}\)-dependent glucose uptake were determined in steers. Glucose infusion resulted in a slight depression (\( P < 0.10 \)) in dietary energy intake (313.5 vs. 306.5 kcal/(kg BW\(^{0.75} \)) d\(^{-1} \)); however, total energy intake (diet plus infusate) did not differ between carbohydrate-infusion treatments. Small intestinal length and empty wet weight averaged 30.4 \( \pm \) 2.8 m and 3.7 \( \pm \) 0.5 kg, respectively, and were not affected by treatment (\( n = 40 \)). Because of the consistency of the replication of data among blocks and one missing observation in Block 8, we only prepared and analyzed brush border membrane vesicles (BBMV) from the intestinal samples of 35 of the 40 steers (seven of the eight blocks). Therefore, \( n = 7 \) for the small intestinal samples assayed for SGLT1 activity.

To assess the effectiveness of membrane isolation enrichments, relative protein levels of SGLT1, GLUT2, and alkaline phosphatase were determined for a subset of the samples: five individual intestinal sampling sites of five steers (Table 2). Enrichments based on maltase activity are included in Table 3. Protein-based enrichment values that were more than three standard deviations from the mean were not used in the analysis (two SGLT1 and GLUT2; three alkaline phosphatase). The distal jejunum and ileum sections were not used for the alkaline phosphatase enrichment because these sections lacked a measurable signal. The highest enrichment of the three proteins used as membrane markers was demonstrated by SGLT1 (Table 2). According to
Table 3. Effects of control (W), twice maintenance energy intake (2M), ruminal starch infusion (RS), abomasal starch infusion (AS), or abomasal glucose (AG) infusion on changes in maltase activity, enrichment, and sodium-dependent glucose uptake by treatment in steers

<table>
<thead>
<tr>
<th>Treatments</th>
<th>W</th>
<th>2M</th>
<th>RS</th>
<th>AS</th>
<th>AG</th>
<th>SEM&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Treatment effect&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate maltase&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23</td>
<td>23</td>
<td>26</td>
<td>34</td>
<td>38</td>
<td>3.0</td>
<td>0.001&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vesicle maltase</td>
<td>127</td>
<td>104</td>
<td>116</td>
<td>119</td>
<td>146</td>
<td>18.7</td>
<td>0.16</td>
</tr>
<tr>
<td>Enrichment&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.7</td>
<td>4.9</td>
<td>5.4</td>
<td>4.9</td>
<td>4.2</td>
<td>0.63</td>
<td>0.41</td>
</tr>
<tr>
<td>Sodium-dependent glucose uptake&lt;sup&gt;e&lt;/sup&gt;</td>
<td>18.24</td>
<td>16.65</td>
<td>19.71</td>
<td>18.19</td>
<td>19.26</td>
<td>3.94</td>
<td>0.98</td>
</tr>
<tr>
<td>SGLT1 abundance&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.05</td>
<td>7.87</td>
<td>0.95</td>
<td>6.09</td>
<td>3.19</td>
<td>8.29</td>
<td>0.80</td>
</tr>
</tbody>
</table>

<sup>a</sup>Probability of larger F.

<sup>b</sup>n = 35.

<sup>c</sup>Maltaise specific activity (SA); nmol of glucose·mg of protein incubated<sup>-1</sup>/min of incubation<sup>-1</sup>.

<sup>d</sup>Enrichment = vesicle SA/homogenate SA.

<sup>e</sup>Glucose uptake; pmol of glucose·mg of protein incubated<sup>-1</sup>/s·mg of protein incubated<sup>-1</sup> of incubation.

<sup>f</sup>Na<sup>+</sup>-dependent glucose cotransporter 1 abundance in vesicles; densitometric values normalized to the duodenum.

<sup>g</sup>n = 4.

<sup>h</sup>AG different from W (P < 0.02).

Immunoblot analysis, SGLT1 exhibited an apparent molecular weight of 76.8 ± 4.6 kDa (Figure 1). The GLUT2 exhibited two bands, 56.5 ± 2.3 and 58.7 ± 2.7 kDa (Figure 2). Alkaline phosphatase consistently exhibited a 75.4 ± 9.04 kDa band for the duodenum, jejunum 1, and jejunum 2 (Figure 3). The vesicles from the duodenum also displayed an additional alkaline phosphatase band of 99.7 ± 5.0 kDa.

There was an effect of treatment on homogenate maltase specific activity (Table 3; P < 0.001). The AG treatment yielded a higher maltase activity compared with W (P < 0.02). There was no effect of treatment on maltase enrichment. Sodium-dependent glucose uptake averaged 18.4 ± 3.94 pmol glucose/(mg protein·s), and there was no effect of treatment on Na<sup>+</sup>-dependent glucose uptake or SGLT1 abundance.

Maltase-specific activity (Table 4) in both the vesicles and homogenates differed with small intestinal sampling site (quadratic, P < 0.001). Maltase-specific activities, for both vesicles and homogenates, were highest in the mid-intestine and declined in the duodenum and ileum. Sodium-dependent glucose uptake was highest in the duodenum and jejunum 1, intermediate in jejunum 2, and decreased rapidly in jejunum 3 and ileum (linear, P < 0.001).

Because of the lack of treatment effects on SGLT1 activity, the effect of intestinal sampling site on SGLT1 abundance was examined in a subset of animals (22 steers total; five 2M, four AS, four AG, four W, and five control).
Figure 3. Immunoblot analysis of alkaline phosphatase in the brush border membrane vesicles (V) and initial homogenate (H). V1, V2, V3, V4, and V5 correspond to duodenum, jejunum 1, jejunum 2, jejunum 3, and ileum for one steer, respectively; H1 through H5 represent the corresponding homogenates. The molecular weight markers are located to the left of the immunoblot. This blot is a representative of the five blots used to obtain enrichment values. Enrichment values were obtained by dividing the densitometric value for V by the value for H. There were no enrichment values reported for jejunum 3 (V4 and H4) or ileum (V5 and H5), because there was no measurable signal.

Discussion

Brush Border Membrane Vesicle Preparation

Brush border membrane vesicles can be used to study nutrient transport, assess enzyme activity, and identify and quantify various brush border proteins. The similarity of maltase, a brush border enzyme, enrichment of intestinal vesicles in this experiment indicates a consistent enrichment of the intestinal brush-border membrane between treatments and intestinal sections. However, the maltase enrichments obtained were lower than previously reported values, which ranged from 7 to 15 for sheep and 4 to 11 for cattle, respectively (Shirazi-Beechey et al., 1989; Bauer et al., 2001a,b). This discrepancy in enrichment values could either be a result of species differences or lower vesicle maltase activity values in the current study.

Alkaline phosphatase is another enzyme commonly used as a brush-border membrane marker. The alkaline phosphatase enrichments obtained in this study were higher than the corresponding maltase enrichments, but lower than previously reported alkaline phosphatase enrichment values that were based on enzyme activity (Kessler et al., 1978; Shirazi-Beechey et al., 1989; Shirazi-Beechey et al., 1990; Bauer et al., 2001a,b). Enrichments of SGLT1 and GLUT2 (Table 2) demonstrate that brush-border membranes were enriched independent of the basolateral membrane. Previous BBMV studies have used Na⁺K⁺ATPase activity to determine the presence of basolateral membrane in vesicles. Christiansen and Carlsen (1981) reported a 1.5-fold enrichment of Na⁺K⁺ATPase in BBMV vesicles. This value is similar to the average 1.0-fold enrichment of GLUT2 observed in the current study. The 56.5- and 58.7-kDa bands observed in the immunoblots fell within the expected range of 53 to 61 kDa for the particular GLUT2 antibody that was used. The band weight was also similar to the 59-kDa band observed in lamb liver (Gelardi et al., 1999).

Based on recent studies (Kellett and Helliwell, 2000) that have suggested the possible trafficking of GLUT2 to the brush-border membrane in response to glucose in the intestinal lumen, use of GLUT2 as a basolateral membrane marker may be questionable. However, GLUT2 typically exits the brush-border within minutes of tissue excision, as a result of inactivation of PKC βII (Kellett, 2001). In this experiment, the time required

Table 4. Effects of control (W), twice maintenance energy intake (2M), ruminal starch infusion (RS), abomasal starch infusion (AS), or abomasal glucose (AG) infusion on changes in maltase activity, enrichment and sodium dependent glucose uptake along the small intestine of steers

<table>
<thead>
<tr>
<th>Small intestinal site</th>
<th>Effecta</th>
<th>Duodenum</th>
<th>Jejunum 1</th>
<th>Jejunum 2</th>
<th>Jejunum 3</th>
<th>Ileum</th>
<th>SEMb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate maltasec</td>
<td></td>
<td>12</td>
<td>39</td>
<td>36</td>
<td>31</td>
<td>27</td>
<td>3.0</td>
</tr>
<tr>
<td>Vesicle maltase</td>
<td></td>
<td>41</td>
<td>142</td>
<td>158</td>
<td>158</td>
<td>114</td>
<td>12.1</td>
</tr>
<tr>
<td>Enrichmentd</td>
<td></td>
<td>5.1</td>
<td>4.1</td>
<td>4.8</td>
<td>5.7</td>
<td>5.4</td>
<td>0.57</td>
</tr>
<tr>
<td>Sodium-dependent glucose uptakex</td>
<td></td>
<td>36.84</td>
<td>31.04</td>
<td>18.91</td>
<td>3.25</td>
<td>2.01</td>
<td>3.40</td>
</tr>
<tr>
<td>SGLT1 abundanced</td>
<td></td>
<td>1.00</td>
<td>1.94</td>
<td>2.42</td>
<td>8.14</td>
<td>8.39</td>
<td>2.95</td>
</tr>
</tbody>
</table>

aProbability of larger F-value.
bn = 35.
cMaltase specific activity (SA); nmol of glucose-mg of protein incubated⁻¹-min of incubation⁻¹.
dEnrichment = vesicle SA/homogenate SA.
xGlucose uptake; pmol of glucose-mg of protein incubated⁻¹-s of incubation⁻¹.
dNa⁺-dependent glucose cotransporter 1 abundance in vesicles; densitometric values normalized to the duodenum site, n = 23.
to retrieve, measure, weigh, clean, and sample the intestine was approximately 15 min, which is long enough to cause inactivation of protein kinase C βII. Therefore, if GLUT2 was trafficked to the brush-border membrane, it was most likely lost during the tissue retrieval process.

The 9.2-fold enrichment of SGLT1 obtained in the current study demonstrates that we enriched the targeted protein. Additionally, the 76.8-kDa band observed on the immunoblots was within the 75- to 78-kDa band range typically found for SGLT1 in ruminant intestinal tissue (Shirazi-Beechey et al., 1991; Wood et al., 2000).

Sodium–Glucose Cotransporter Protein Abundance

There was no effect of treatment on SGLT1 abundance in the BBMV (Table 3); however, SGLT1 abundance increased from the duodenum to the ileum (Table 4). The lack of a treatment effect on SGLT1 protein abundance in this experiment is not surprising as there was no treatment effect on SGLT1 activity. Past research demonstrated a positive correlation between SGLT1 protein and activity in sheep (Dyer et al., 1994). It is also not surprising that SGLT1 activity decreased distally throughout the small intestine, as this has been reported previously (Bauer et al., 2001a,b). However, it is surprising that SGLT1 protein abundance increased as activity decreased distally in the small intestine. Although the densitometric values were not reported, previous immunoblot analysis of bovine small intestinal vesicle preparations have demonstrated a strong SGLT1 signal from membrane preparations of the jejunum and ileum, as opposed to a faint signal from the duodenal membrane preparation (Zhao et al., 1998). Because the amount of vesicle protein loaded on the gel was the same among tissues, the difference in immunoblot signal intensity indicates that more SGLT1 protein is present in the jejunum and ileum vs. the duodenum.

Conventional thinking is that “animals don’t build and maintain structures in excess of what they need” (Taylor and Weibel, 1981). In keeping with that mode of thinking, SGLT1 may have a role in the distal intestine besides glucose transport. It has been suggested that SGLT1 can actively transport water along with Na+ and glucose or passively channel water through the brush-border membrane (Wright and Loo, 2000); thus, the primary function of SGLT1 in the distal intestine could be water absorption. However, this is speculative because it is unclear whether water transport can be uncoupled from glucose transport.

An alternative hypothesis would be that the SGLT1 protein present in the distal small intestine is not as active as the SGLT1 protein found in the proximal intestine. Perhaps the protein is less active because ruminants evolved consuming forage, and therefore, there was no need for forage-fed ruminants to maintain SGLT1 function in the distal small intestine. Additionally, it has been suggested there may be differences in the rate of enzyme reaction (V_max) and Michaelis-Menten constant (K_m) of SGLT1 along the intestine. Okine et al. (1997) demonstrated that the V_max of Na+-dependent glucose uptake decreased along the small intestine, whereas the K_m increased. A lower V_max for SGLT1 in the distal intestine might explain the decreased transporter activity; however, the increased protein abundance with decreasing SGLT1 activity suggests that much of the protein is either less active or inactive.

Sodium–Glucose CoTransport Activity

It has been demonstrated that glucose transport can be upregulated in response to the presence of luminal substrate, because greater glucose uptake was induced by infusing a 30 mM D-glucose solution into the proximal intestine of adult sheep for 4 d. Glucose uptake was 40 to 80 times greater in BBMV isolated from infused sheep (310 ± 40 pmol/[mg protein-s]) than control sheep (4 ± 1.5 pmol/[mg protein-s]; Shirazi-Beechey et al., 1991). More recently, Bauer et al. (2001b) observed an increase in Na+-dependent glucose uptake in response to luminal α-linked glucose for sheep, but they did not obtain the same magnitude of increase in glucose uptake as observed in the previous study (Shirazi-Beechey et al., 1991). There was only a 2.1-fold increase in glucose uptake for postruminal vs. ruminal starch hydrolysate infusion (Bauer et al., 2001b). The lower magnitude of upregulation of Na+-dependent glucose uptake may partially be a reflection of the higher amount of uptake for the control animals in the study of Bauer et al. (2001b) as opposed to Shirazi-Beechey et al. (1991): 47.2 and 4 pmol/[mg protein-s], respectively. Bauer et al. (2001b) hypothesized that hydrolysis of α-linked glucose in the small intestine limited glucose availability, and therefore restricted up-regulation of Na+-dependent glucose uptake. In contrast, the results of the current study fail to support this hypothesis, as supplying glucose abomasally did not increase glucose transport as compared with the other treatments or control in cattle.

In the current study, glucose transport activity differed by intestinal site, but not by treatment (Table 2). Qualitatively, these results are similar to a separate study by Bauer et al. (2001a), in which no difference between glucose uptake for steers infused ruminally (240 ± 26 pmol/[mg-s]) or postruminally (204 ± 24 pmol/[mg-s]) with starch hydrolysate was observed. Quantitatively, lower glucose uptake (18.41 ± 3.40 pmol/[mg-s]) was observed in the current study (Table 4). However, glucose uptakes in the current study were higher than uptakes reported for sheep or cows consuming a forage-based diet, 1.4 ± 0.42 and 0.52 ± 0.3 pmol/[mg-s]), respectively (Wood et al., 2000). The fact that there was measurable glucose uptake in the 2M and W treatment steers was also in agreement with data that suggested that animals consuming a forage-based diet...
still have the ability to actively transport glucose from the intestinal lumen, despite the low small intestinal glucose concentrations associated with such diets (Bauer et al., 1995).

The decrease in Na⁺-dependent glucose uptake along the intestinal tract was similar to previously reported data in lambs, where activity in the proximal intestine was higher than the distal intestine: 233 ± 100 and 90 ± 27 pmol/(mg protein·s), respectively (Shirazi-Beechey et al., 1989). This data is in agreement with in vivo data that has demonstrated greater glucose disappearance when glucose is infused duodenally vs. midjejunally, leading to the conclusion that glucose absorption occurs primarily in the proximal-half of the small intestine (Krehbiel et al., 1996).

As previously observed by Bauer et al. (2001b), the pattern of glucose uptake did not match that of maltase activity. Glucose uptake declined along the tract, whereas maltase activity increased up to the second jejunal section and then remained relatively elevated through the ileum. It has been suggested that the capacity for glucose absorption from the small intestine exceeds maltase activity (Wright et al., 1966; Pehrson et al., 1981). However, a comparison of vesicle maltase activity with vesicle SGLT1 activity indicates that maltase activity was approximately 100-fold greater when evaluated across all intestinal sites (Table 3), and from 18- to more than 900-fold greater when comparing within sites in the small intestine (Table 4). Such a disparity in maltase and SGLT1 activities may suggest that alternatives to SGLT1 exist for glucose exit from the bovine small intestine.

Homogenate maltase activity was affected by treatment in the current experiment. The homogenate fraction is considered to be more representative of the small intestine because it is obtained directly from the diced tissue filtrate, prior to the divergent cation precipitation and the differential centrifugation. Kreikemeier et al. (1990) observed that maltase distribution was not affected by diet, but that total hydrolytic activity of the intestine tended to increase with increasing ME intake, largely because of a longer small intestine. In contrast, McNeill et al. (1974) observed an increase in maltase activity in small intestinal biopsies taken from lambs receiving duodenal glucose infusions. In the current study, AG treatment delivered the most glucose to the small intestine, followed by the AS treatment. The RS, 2M, and W treatments delivered very little glucose to the small intestine, corresponding directly with the maltase activities observed and suggesting that enhanced maltase activity responds to luminal substrate but not energy intake.

Conclusion

Sodium-dependent glucose uptake capacity and total glucose uptake capacity did not respond to glucose or starch infusion, whereas maltase activity increased in response to glucose infusion. Sodium-dependent glucose cotransporter 1 protein was found throughout the intestinal tract, despite very low levels of Na⁺-dependent glucose uptake in the distal intestine. Based on the observation that glucose uptake was not upregulated in response to increased luminal free or α-linked glucose, glucose uptake either is not limiting to small intestinal glucose absorption or is not inducible in cattle. The inverse relationship between glucose uptake and Na⁺-dependent glucose cotransporter 1 abundance may reflect a difference in Na⁺-dependent glucose cotransporter 1 regulation or function for the proximal vs. the distal intestinal tract. Further studies designed to establish the relationship between Na⁺-dependent glucose cotransporter 1 activity and abundance in response to glucose, as well as Na⁺ and water along the intestine, may reveal alternative roles of Na⁺-dependent glucose cotransporter 1 in the small intestine.

Implications

Starch assimilation into body energy stores is more energetically efficient when glucose is absorbed in the small intestine vs. VFA absorption in the rumen. Thus, it is advantageous to understand any possible limits to glucose absorption in the small intestine. Results of this study suggest that glucose transport is either not limiting to glucose absorption in the small intestine of cattle or that cattle lack the ability to substantially upregulate glucose uptake in response to increased dietary load. In contrast, maltase activity increased in response to increased luminal substrate. Overall, it seems that diet has little effect on the ability of cattle to transport glucose from the small intestine.

Literature Cited
