Glucose Transporter Gene Expression in Bovine Mammary Gland

Feng-Qi Zhao*2, Erasmus K. Okine†3, and John J. Kennelly*

*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada T6G 2P5 and †Western Forage/Beef Group, Lacombe Research Centre, Lacombe, Alberta, Canada T4L 1W1

ABSTRACT: Transport of glucose across the plasma membrane of mammary epithelial cells is believed to be a passive process of facilitated diffusion mediated by facilitative glucose transporter(s). This article presents three lines of evidence that indicate the expression of sodium/glucose cotransporter (SGLT1) in the mammary gland of lactating and nonlactating cows. First, transcripts of SGLT1 mRNA ranging in size from 1.5 to 5.2 kb were detected in polyadenylated RNA preparations of mammary glands of lactating and nonlactating cows. Second, SGLT1 cotransporter protein was also detected in plasma membrane preparations of mammary glands of lactating cows. Third, partial amino acid sequence deduced from the reverse transcriptase-PCR fragment of SGLT1 from bovine mammary glands was similar to the sequence reported for ovine SGLT1. We conclude that mammary gland expression of SGLT1 mRNA and protein suggests that an active glucose transport system may be involved in glucose transport and metabolism in the mammary gland of dairy cows. However, the physiological significance of the expression of SGLT1 in mammary gland remains unknown.

Key Words: Bovidae, Mammary Glands, Glucose, Gene Expression

Introduction

Glucose is the main precursor of lactose synthesis in mammary gland epithelial cells (Neville et al., 1983). However, a lactating mammary gland does not synthesize glucose from other precursors due to the lack of glucose-6-phosphatase (Threadgold and Kuhn, 1979) and is, therefore, dependent on the blood supply for its glucose needs. A steep concentration gradient of glucose is established across the plasma membrane: 3.0 to 3.5 mM in plasma to .1 to .3 mM within the cell (Faulkner et al., 1981). This evidence has led to the belief that transport of glucose across the plasma membrane of mammary epithelial cells may only be a passive process of facilitated diffusion (Delaquis et al., 1993). Indeed, Zhao et al. (1996) reported that GLUT1 may be the predominant facilitative glucose transporter in a lactating bovine mammary gland. There are reports of both active and passive transport of glucose across the small intestine (Huntington, 1997; Zhao et al., 1998). However, there is little or no information on whether there may be an active component of transport of both glucose and UDP-galactose into the Golgi apparatus of the mammary gland for lactose synthesis. The sodium/glucose cotransporter (SGLT1) has been reported to be located at the brush border membrane of intestinal epithelial cells and kidney and is involved in active glucose transport using the electrochemical gradient of Na+ generated by Na+,K+ ATPase (Silverman, 1991; Wright, 1993).

In this paper, we report on the detection of sodium-dependent glucose cotransporter mRNA and protein and the partial amino acid sequence of SGLT1 from a reverse transcriptase-PCR fragment of bovine mammary glands.
Materials and Methods

Animals, Tissue Removal, and Plasma Membrane Preparation

The cows were cared for according to the guidelines of the Canadian Council on Animal Care. Four primiparous, midlactation Holstein cows (100 to 200 d of lactation; 635 ± 64 kg BW), tethered in stalls with free access to water, were used as tissue donors. The diet of these cows was formulated to satisfy the nutrient requirements of a Holstein cow weighing 600 to 650 kg and producing 30 kg of milk daily. The diet was a 50:50 forage:concentrate mixture of barley, canola and soybean meal, ground corn, molasses, and mineral-vitamin premix (Okine et al., 1997). Mammary glands were collected from two adult Sprague-Dawley rats (Okine et al., 1997). Mammary glands were collected from two adult Sprague-Dawley rats fed rat chow and one 3-wk-old calf fed milk. All tissues of interest were removed within 10 min after the slaughter of the animals and either frozen in liquid nitrogen for RNA preparation or chilled immediately on ice during transportation to the laboratory for plasma membrane preparations.

The procedures of isolation of plasma membrane from bovine mammary gland were modified from Kanno et al. (1982). Tissue was finely minced with scissors into pieces less than 5 mm³ and rinsed three to four times with three volumes of wash buffer (.25 M sucrose, 20 mM Tris-HCl [pH 7.4], 1 mM EDTA, .5 mM PMSF, and 1 μg/mL pepstatin A) by sedimentation at 500 × g for 3 min at 2°C. Portions corresponding to about 50 g of minced tissue were rough disrupted in 200 mL of homogenization buffer (wash buffer, plus 1 μg/mL leupeptin, 1 μg/mL chymostatin, 1 μg/mL antipain, and 1 μg/mL aprotinin) in a blender twice for a total of 1 min at medium speed. The homogenate was filtered through one layer of prewetted surgical gauze. The filtrate was then homogenized by repeated passage through a 27-gauge needle, and stored in liquid nitrogen.

Brush-border membrane vesicles of rat jejunum and calf ileum were prepared from the two adult Sprague-Dawley rats and one 3-wk-old calf, as described by Zhao et al. (1998) and used as positive controls for protein blot analyses.

The activity of 5′-nucleotidase was used as a plasma membrane marker and was determined by measuring the rate of release of inorganic phosphate as described by Huang and Keenan (1972). Protein concentrations were measured with the Bradford dye-binding assay (Bradford, 1976) using the BIO-RAD protein Assay Kit (Bio-RAD, Richmond, CA) and BSA as a standard.

mRNA Blotting

Total RNA was isolated from tissue samples with a guanidinium thiocyanate/phenol/chloroform extraction procedure (Chomczynski and Sacchi, 1987). Polyadenylated RNA (poly A⁺ RNA) was isolated from total RNA using oligo(dT) cellulose chromatography (Jacobson, 1987). The RNA was electrophoresed on denaturing 1% agarose/0.66 M formaldehyde gels and transferred to nylon membranes (Bio-Rad) by upward capillary diffusion. After electrophoresis and transfer, membranes were baked under vacuum at 80°C for 2 h.

Membranes were prehybridized for 2 h at 50°C in a medium containing 60% (vol/vol) formamide, 1 × SSPE (.18 M NaCl, .01 M sodium phosphate at pH 7.4, and 1 mM EDTA), .5% (wt/vol) nonfat dried milk (Carnation Ltd., Ontario, Canada), 10% (wt/vol) dextran sulfate, 1% (wt/vol) SDS, 500 μg/mL of salmon testes DNA, and 200 μg/mL of yeast tRNA. After prehybridization, hybridization was carried out for 16 to 18 h at 50°C in fresh buffer containing [3²P]-labeled riboprobes (10⁶ cpm/mL). The SGLT1 antisense riboprobe was generated from a plasmid that contained a 1.4-kb fragment of lamb intestinal SGLT1 (corresponding to amino acids 207 to 664) (Wood et al., 1994). Membranes were then rinsed briefly in 2× SSC (1× SSC is .15 M NaCl and .015 M sodium citrate at pH 7.0), washed for 15 min at 22°C in 2× SSC containing .1% SDS, stringently washed at 70°C for 35
min in .2× SSC containing 1% SDS, and rinsed briefly in .2× SSC. Autoradiography was performed for 4 to 72 h at −70°C with intensifying screens.

**Protein Blotting**

Membranes were resuspended in Laemmli sample buffer (Laemmli, 1970) and resolved on 10% SDS-PAGE using a Mini-protein II Electrophoresis Cell (Bio-Rad). Equal protein loading was ensured by diluting all samples to the same protein concentration and then using the same volume for each lane. The proteins were electrophoretically transferred to nitrocellulose filters (BA 85; Schleicher and Schuell, Dassel, Germany). Transfer was assessed by staining the nitrocellulose with ponceau S. In addition, protein markers (Rainbow markers; Amersham, Arlington Heights, IL) were used as molecular mass standards and to assess the efficiency of the transfer.

The blot was blocked overnight at 4°C in TBS (20 mM Tris [pH 7.4] and 137 mM NaCl) containing 5% nonfat dried milk and incubated for 1.5 h at room temperature in TBS, .5% nonfat dry milk, with a 1:1,000 dilution of SGLT1 antiserum. The filter was then washed twice at room temperature for 15 min in TBS and incubated for 1 h at room temperature in TBS, .5% nonfat dried milk, with a 1:2,000 dilution of goat anti-rabbit IgG conjugated to horseradish peroxidase (Calbiochem, La Jolla, CA). The immune complex was detected using the Amersham ECL Western blotting system following the manufacturer’s instructions. The blot was autoradiographed (Hyperfilm ECL; Amersham International, Buckinghamshire, England) and quantified with scanning densitometry (Imaging Densitometer GS-670; Bio-Rad Laboratories, Missisauga, Ontario, Canada).

The primary antibody used was a polyclonal antibody raised against a synthetic peptide (STLFTMDIYTKIRKKASEK) corresponding to residues 402 to 419 of the rabbit SGLT1 sequence (Hediger et al., 1987). Specificity of this antibody has been demonstrated by Lescalet-Matys et al. (1993).

**Reverse Transcriptase-PCR and Sequencing of SGLT1**

A partial bovine SGLT1 sequence was amplified by reverse transcriptase-PCR using two primers (5’ primer, GTTGGCCTGACCAATCGCTACC; 3’ primer, CCAAGTAACTGGATGACTGGAT) that were generated based on ovine SGLT1 sequence (Wood et al., 1994). The first strand cDNA was prepared from .5 μg of DNase-digested polyadenylated RNA from two early-lactation bovine mammary glands using 3’ primer and SuperScript RNase H Reverse Transcriptase (Gibco-BRL, Life Technologies, Burlington, Ontario, Canada). This cDNA was directly PCR amplified in a final volume of 100 μL using .5 μM of the 5’ and 3’ primers. The PCR amplifications were done with five units of Taq-polymerase (Gibco-BRL) by 35 cycles of 45 s at 94°C, 30 s at 55°C, and 90 s at 72°C. The amplified DNA was gel purified and reamplified under same conditions as above. The resulting DNA was finally precipitated in 11% polyethylene glycol (PEG8000) and sequenced using an automatic DNA sequencer (Perkin Elmer Model 373 A sequencer, Foster City, CA).

**Results**

**Expression of SGLT1 mRNA in Bovine Mammary Gland**

Hybridization of polyadenylated RNA blots under stringent conditions with the SGLT1 antisense riboprobe yielded more than four transcripts from

Figure 1. Northern blotting of SGLT1 mRNA in mammary gland (BM) of lactating cows. Total RNA (T, 30 μg/lane) and polyadenylated RNA (A+, 10 μg/lane) were pooled samples isolated from two lactating cows or two adult Sprague-Dawley rats. The total RNA from rat duodenum (RD) and jejunum (RJ) was used as positive control. The total RNA from rat liver (RL) and the mammary total RNA depleted of polyadenylated RNA (A−, 30 μg) were used as negative controls. The transcripts of SGLT1 mRNA were also detected in bovine kidney (BK). The sizes (k = kilobases) of the hybridizing transcripts are indicated.
lactating bovine mammary gland (Figure 1). The sizes of these transcripts ranged from 1.5 to 5.2 kb and are similar to those reported from sheep (Lescale-Matys et al., 1993). Transcripts of SGLT1 were also detected in rat duodenum and jejunum and bovine kidney, but with different size patterns (Figure 1). Transcripts of SGLT1 were essentially absent in mammary gland total RNA depleted of polyadenylated RNA. On the other hand, transcripts of SGLT1 mRNA (poly A+) were detected in mammary gland from nonlactating and lactating cows (Figure 2).

Expression of SGLT1 Protein in Bovine Mammary Gland

The enrichment of the 5′-nucleotidase activity in the F1, F2, and crude membrane fractions were 5.9 ± .1, 9.0 ± .6, and 3.2 ± .2 higher, respectively, relative to the homogenate preparation. Immunoblot analysis detected SGLT1 protein in the F1, F2, and crude membrane fractions, but not in the mammary homogenate (Figure 3). However, quantification of the 60-kDa SGLT1 protein band indicated that the protein abundance in these membrane fractions (8.81 ± 2.1) was much less than the abundance in the rat jejunum and calf ileum (16.0 ± 3.1 and 17.02 ± 2.8 O.D. × mm², respectively). The abundance was highest in the F2 fraction congruent with the level of enrichment of 5′-nucleotidase activity in mammary membrane preparations. The estimated molecular weight of the protein in the SGLT1 protein band (approximately 60 kDa) in the mammary gland was less than that detected in the calf ileum and rat jejunum.

Partially Sequencing of SGLT1 from Bovine Mammary Gland

To further confirm the expression of SGLT1 in the mammary gland, we amplified and sequenced the partial SGLT1 cDNA in the mammary gland by PCR using primers generated based on ovine SGLT1 cDNA (Wood et al., 1994). In the 283 base pairs, only five base pairs were different between the bovine and ovine species (Figure 4A). However, all the differences were detected at silent sites and did not change the amino acid sequence of this region, which includes the 19 amino acids to which the primary antibody used in this study was raised (Figure 4B).
Figure 4. A) Partial sequence of mammary SGLT1 cDNA generated from pooled samples from two lactating cows by PCR compared with the corresponding sequence of ovine SGLT1. The primer sequences are shown in italic and the sequence differences between two species are shown in boldface. B) The partial amino acid sequence of SGLT1 deduced from partial bovine mammary SGLT1 cDNA is shown to be exactly the same as the corresponding sequence of ovine SGLT1 protein. The 19 amino acids to which the primary antibody was raised are shown in boldface.

Discussion

Several lines of evidence in this study indicate that a lactating bovine mammary gland expresses the sodium-dependent glucose transporter, SGLT1. First, SGLT1 mRNA was detected in mammary glands from lactating and nonlactating cows. Second, the SGLT1 protein was detected in the plasma membrane preparations of lactating mammary glands. Third, partial amino acid sequence of bovine SGLT1 deduced from a reverse transcriptase-PCR fragment from bovine mammary gland was similar to the ovine SGLT1, indicating that the same peptide sequence is conserved in bovine SGLT1.

It has generally been accepted that the transport of glucose across the plasma membrane of mammary epithelial cell is a passive process of facilitated diffusion, because of the steep concentration gradient of glucose across the plasma membrane. Indeed, Delaquis et al. (1993) reported a Na⁺-independent glucose uptake by bovine mammary epithelial (MAC-T) cells. Zhao et al., 1993, 1996) used five human facilitative glucose transporter cDNA in mRNA blotting analysis of bovine tissues and reported that GLUT1 mRNA was present at relatively high level in mammary gland from lactating cows. This finding was consistent with previous studies with lactating rat mammary gland that used quantitative protein blotting and cytochalasin B-binding and revealed that GLUT1 may constitute the major glucose transporter species in the plasma membranes of rat mammary gland epithelial cells (Madon et al., 1990). Thus, our observation that mammary glands express the sodium-dependent glucose transporter, SGLT1, which is an active transport system and uses the electrochemical gradient of Na⁺ to transport glucose against its concentration gradient, was unexpected. Sodium-dependent glucose transporter mRNA and protein have been found in the brush-border membranes of the small intestine and kidney epithelial cells and have been reported to be responsible for the absorption of glucose from the lumen of the small intestine and proximal tubule of the kidney (Wright, 1993; Zhao et al. 1998). The expression of SGLT1 mRNA and detection of the protein in the mammary gland raise the important questions of the subcellular localization and the physiological significance of SGLT1 in the mammary gland.

One possible subcellular localization of SGLT1 in the mammary gland may be the Golgi membrane. Lactose is synthesized in the Golgi apparatus using glucose and UDP-galactose, which is also derived from
glucose in the cytosol. The mechanisms by which the glucose crosses the membranes of Golgi vesicles to reach the site of lactose synthesis remains unclear. It is likely that the glucose concentration in the lumen of Golgi apparatus may sometimes be higher than that in the cytosol so that SGLT1 may be required for glucose translocation from the cytosol into Golgi lumen.

Detection of SGLT1 protein in our mammary plasma membrane preparations could be due to the presence of Golgi membrane in the mammary gland fractions isolated with our methods. Indeed, the 5′-nucleotidase enrichments were only 5.9 and 9.0 times in F1 and F2 fractions relative to the homogenate, respectively, and may indicate that the fractions were not entirely devoid of fragments of Golgi apparatus. Membranes isolated with similar methods in other studies have shown likely Golgi membrane contamination, as indicated by extensive amounts of galactosyltransferase activity (Shin et al., 1975; Huggins and Carraway, 1976). In addition, the size of the SGLT1 protein band (approximately 60 kDa) in the mammary gland was smaller than the size (69 kDa) detected in the calf ileum and rat jejunum. However, differences in the size of the SGLT1 protein may be the result of different glycosylation or phosphorylation of SGLT1 in different tissues.

Despite the equivocal nature of the possible location of the SGLT1 detected in this study, the possibility that SGLT1 is located in the plasma membrane of the mammary epithelial cells cannot be entirely ruled out. Data from White et al. (1980) and Kuhn (1983) indicate that sugar uptake into Golgi vesicles occurs via passive, nonstereospecific proteinaceous pores, because the Golgi membrane discriminates poorly between different sugars of Mr < 300. Thus, possible contamination of our plasma membrane fractions with Golgi membranes does not negate the probability that SGLT1 may be located in the plasma membrane of the mammary epithelial cells.

Implications

The mammary glands of lactating and nonlactating cows seem to express the active sodium-dependent glucose transporter (SGLT1). The mammary expression of SGLT1 mRNA and protein suggest that an active glucose transport system may be involved in the metabolism of glucose in the mammary gland in addition to the passive mechanisms of glucose transport. Further experiments are required for the intracellular location and potential physiological roles of SGLT1 in the mammary gland.

Literature Cited


