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Expression and Regulation of Glucose Transporters in the Bovine Mammary Gland[1]

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ABSTRACT

Glucose is the primary precursor for the synthesis of lactose, which controls milk volume by maintaining the osmolarity of milk. Glucose uptake in the mammary gland plays a key role in milk production. Glucose transport across the plasma membranes of mammalian cells is carried out by 2 distinct processes: facilitative transport, mediated by a family of facilitative glucose transporters (GLUT); and sodium-dependent transport, mediated by the Na^+/glucose cotransporters (SGLT). Transport kinetic studies indicate that glucose transport across the plasma membrane of the lactating bovine mammary epithelial cell has a K_m value of 8.29 m*M* for 3-*O*-methyl-D-glucose and can be inhibited by both cytochalasin-B and phloretin, indicating a facilitative transport process. This is consistent with the observation that in the lactating bovine mammary gland, GLUT1 is the predominant glucose transporter. However, the bovine lactating mammary gland also expresses GLUT3, GLUT4, GLUT5, GLUT8, GLUT12, and sodium-dependent SGLT1 and SGLT2 at different levels. Studies of protein expression and cellular and subcellular localizations of these transporters are needed to address their physiological functions in the mammary gland. From late pregnancy to early lactation, expression of GLUT1, GLUT8, GLUT12, SGLT1, and SGLT2 mRNA increases from at least 5-fold to several hundred-fold, suggesting that these transporters may be regulated by lactogenic hormones and have roles in milk synthesis. The GLUT1 protein is detected in lactating mammary epithelial cells. Its expression level decreases from early to late lactation stages and becomes barely detectable in the nonlactating gland. Both GLUT1 mRNA and protein levels in the lactating mammary gland are not significantly affected by exogenous bovine growth hormone, and, in addition, GLUT1 mRNA does not appear to be affected by leptin.

Key words: glucose transporter, glucose uptake, lactation, mammary gland

INTRODUCTION

In lactating animals, providing glucose for the mammary gland is a metabolic priority because glucose is the primary precursor for lactose synthesis in the mammary epithelial cell. Because lactose maintains the osmolarity of milk, the rate of lactose synthesis serves as a major factor influencing milk volume (Neville et al., 1983; Cant et al., 2002). In addition to its importance as the precursor for lactose synthesis, glucose is also used for the generation of ATP and the cofactor nicotimamide dinucleotide phosphate and is used as a substrate for protein, lipid, and nucleotide synthesis. The mammary gland itself cannot synthesize glucose from other precursors because of the lack of glucose-6-phosphatase (Scott et al., 1976; Threadgold and Kuhn, 1979). Therefore, the mammary gland is dependent on the blood supply for its glucose needs. In a lactating cow, 72 g of glucose is required to produce 1 kg of milk (Kronfeld, 1982). Therefore, in a cow producing 40 kg of milk per day the mammary gland is required to take up about 3 kg of glucose daily. Indeed, mammary gland uptake can account for as much as 60 to 85% of the total glucose that enters the blood (Annison and Linzell, 1964; Chaiyabutr et al., 1980; Sunehag et al., 2002, 2003).

Direct and indirect techniques demonstrate that a steep glucose concentration gradient exists across the basal plasma membrane of mammary epithelial cells, from 2.0 to 5.0 m*M* in plasma to 0.1 to 0.5 m*M* in the cell (Faulkner et al., 1981; Cherepanov et al., 2000). The intracellular glucose concentration is significantly lower than the K_m for the rate-limiting enzyme in lactose synthesis, lactose synthase (Faulkner and Peaker, 1987). Therefore, glucose transport across the plasma membrane may be a rate-limiting step in milk synthesis, and several observations support this theory. Lactose synthesis and milk yield show a linear or positive correlation with glucose uptake in the mammary gland of goats and cows (Kronfeld, 1982; Nielsen and Jakobsen, 1993; Hurtaud et al., 2000; Kim et al., 2001; Niel-

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sen et al., 2001; Cant et al., 2002; Huhtanen et al., 2002). The galactopoietic effect elicited by exogenous bovine growth hormone (**GH**) requires increased glucose uptake by mammary cells (Davis et al., 1988; Mepham et al., 1990; Faulkner, 1999). Milk lactose synthesis and milk yield decrease in cows treated with phlorizin, a potent inhibitor of intestinal glucose absorption and renal glucose reabsorption by inhibiting the Na^+/D-glucose cotransporter (Bradford and Allen, 2005). Regulation of glucose transport in the bovine mammary gland as well as in other tissues also serves a key role in maintaining glucose homeostasis during lactation (for reviews, see Bell, 1995; Bell and Bauman, 1997).

GLUCOSE TRANSPORT AND GLUCOSE TRANSPORTERS IN MAMMALIAN CELLS

The plasma membranes of virtually all mammalian cells possess one or more transport systems to allow glucose movement either into or out of the cells. In mammals, blood glucose levels are maintained within a narrow range by homeostatic mechanisms, and most cells take up glucose by a passive, facilitative transport process, driven by the downward glucose concentration gradient across the plasma membrane (Widdas, 1988). This facilitative transport is inhibitable by cytochalasin-B or phloretin. It is believed that only in the epithelial cell brush border of the small intestine and the kidney proximal convoluted tubules is glucose absorbed or reabsorbed by an active mechanism that uses the downward sodium concentration gradient to transport glucose against its electrochemical gradient. This sodium-dependent transport can be inhibited by phlorizin. The facilitative and sodium-dependent glucose transport systems are mediated by 2 distinct families of glucose transporters (Table 1).

Facilitative Glucose Transporters

Saturable, stereoselective, bidirectional, and energy-independent facilitated diffusion is mediated by the facilitative glucose transporters (solute carriers SLC2A, protein symbol GLUT). Thus far, 13 functional facilitative glucose transporter isoforms have been cloned, characterized and designated as GLUT1–GLUT12 (based on the chronological order of publication) and H^+/myo-inositol cotransporter (**HMIT**). A genomic-wide GenBank search indicated that these 13 isoforms may represent all facilitative glucose transporter members in humans (Joost and Thorens, 2001). These transporters are structurally conserved and related, consisting of 12 transmembrane domains with both the amino- and carboxy-terminals located in the cytoplasm, and an N-glycosylation site located on either the first or ninth extracellular loop. The known kinetic properties, major sites of expression, and proposed function of each of these transporters in human and rodent tissues are summarized in Table 1. Their tissue-specific distribution, distinct kinetic properties, and differential regulation by ambient glucose and hormones, especially insulin, reveal that each transporter isoform plays a specific role in glucose uptake in various tissues and in glucose homeostasis (Wood and Trayhurn, 2003).

The glucose transporters GLUT1 to GLUT5 have been extensively studied. GLUT1 has been ubiquitously detected in cells and tissues, including the mammary gland (Madon et al., 1990; Burant et al., 1991; Zhao et al., 1999). In many tissues in which it is expressed, GLUT1 is concentrated in the cells of blood–tissue barriers (Cornford et al., 1994). Because of the ubiquitous distribution and cellular localization, GLUT1 is considered to be the primary transporter responsible for basal glucose uptake. GLUT2 is involved in the release of hepatic glucose, in the release of absorbed and reabsorbed glucose in the small intestine and kidney, respectively, and in the regulation of insulin secretion from β-cells. GLUT3 plays a major role as the brain neuronal glucose transporter. GLUT4 mediates insulin-stimulated glucose uptake in skeletal muscle and adipose tissues (Holman and Sandoval, 2001). GLUT5 may participate in the uptake of dietary fructose from the lumen of the small intestine.

GLUT6 to GLUT12 and HMIT are the most recently cloned facilitative glucose transporter isoforms and have been characterized to a lesser extent. GLUT6 mRNA is primarily expressed in the spleen, peripheral leukocytes, and brain (Doege et al., 2000a), but its activity is still unclear. GLUT7 was cloned from human small intestinal tissue and was also found to be expressed in the colon, testis, and prostate (Li et al., 2004); it transports both glucose and fructose with high affinity (Li et al., 2004). GLUT8 is postulated to be another insulin-regulated glucose transporter because it was found to be responsible for insulin-stimulated glucose uptake in the blastocyst (Carayannopoulos et al., 2000). It may also be involved in providing glucose for DNA synthesis in male germ cells because it is highly expressed in the testis, and this expression is markedly inhibited by estrogen treatment (Doege et al., 2000b). GLUT9 is found primarily in the kidney and liver (Phay et al., 2000). GLUT10 has the highest levels of expression in the liver and pancreas (McVie-Wylie et al., 2001), and because human GLUT10 is localized to chromosome 20q12-q13.1, one of the genomic loci associated with non-insulin-dependent diabetes mellitus, it may be a candidate for a susceptibility marker for non-insulin-dependent diabetes mellitus. Multiple GLUT11 tis-

Table 1. Summary of the properties of facilitative glucose transporter and Na^+/glucose cotransporter family members

Protein[1]	Major isoform, AA	K_m,[2] mM	Major sites of expression	Proposed function
GLUT1 (Mueckler et al., 1985)	492	6.9	Ubiquitous distribution in tissues and culture cells	Basal glucose uptake; transport across blood-tissue barriers
GLUT2 (Fukumoto et al., 1988)	524	16.2	Liver, islets, kidney, small intestine	High-capacity low-affinity transport
GLUT3 (Kayano et al., 1988)	496	1.8	Brain and nerve cells	Neuronal transport
GLUT4 (Fukumoto et al., 1989)	509	4.6	Muscle, fat, heart	Insulin-regulated transport in muscle and fat
GLUT5 (Kayano et al., 1990)	501	?[3]	Intestine, kidney, testis	Transport of fructose
GLUT6 (Doege et al., 2000a)	507	5	Spleen, leukocytes, brain	
GLUT7 (Li et al., 2004)	524	0.3	Small intestine, colon, testis	Transport of fructose
GLUT8 (Carayannopoulos et al., 2000; Doege et al., 2000b)	477	2.4	Testis, blastocyst, brain, muscle, adipocytes	Fuel supply of mature spermatozoa; insulin-responsive transport in blastocyst
GLUT9 (Phay et al., 2000)	511/540	?	Liver, kidney	
GLUT10 (McVie-Wylie et al., 2001)	541	0.3	Liver, pancreas	
GLUT11 (Doege et al., 2001)	496	?	Heart, muscle	Muscle-specific; fructose transporter
GLUT12 (Rogers et al., 2002)	617	?	Heart, prostate, mammary gland	
HMIT (Uldry et al., 2001)	618/629	?	Brain	H^+/myo-inositol cotransporter
SGLT1 (Hediger et al., 1987)	664	0.2	Kidney, intestine	Glucose reabsorption in intestine and kidney
SGLT2 (Wells et al., 1992)	672	10	Kidney	Los affinity and high selectivity for glucose
SGLT3 (Kong et al., 1993)	660	2	Small intestine, skeletal muscle	Glucose activated Na^+ channel

[1]Pertinent references are shown in parentheses.

[2]Net influx for 2-deoxyglucose.

[3]? = Unknown.

sue-specific splicing variants have been reported to be expressed in a number of tissues, but most abundantly in the skeletal muscle and heart (Wu et al., 2002). GLUT12 was originally cloned from breast cancer cells (Rogers et al., 2002), and its expression has been found in the heart, skeletal muscle, brown adipose tissue, prostate, and mammary glands of both pregnant and lactating rats. Finally, HMIT is expressed predominantly in the brain with specific transport activity for myo-inositol (Uldry et al., 2001).

Na^+/Glucose Cotransporters

The second family, the sodium-dependent glucose transporters, or Na^+/glucose cotransporters (solute carriers SLC5A, protein symbol SGLT), mediate the active, sodium-linked glucose transport process. Concordant with this active transport function, the Na^+/glucose cotransporters are mainly located in the brush-border membranes of epithelial cells in the small intestine and the proximal convoluted tubule of the kidney (Wright and Turk, 2004). Wright and coworkers isolated the first cDNA clone encoding a protein exhibiting all the properties of the small intestinal brush-border transporter and designated it SGLT1 (Hediger et al., 1987). Since the cloning of SGLT1, other isoforms of SGLT genes have been discovered, including SGLT2 (Wells et al., 1992), SGLT3 (Kong et al., 1993), and at least 6 SGLT-related orphan cDNA (Pajor and Wright, 1992; Wright, 2001). These proteins contain several characteristic and conserved sodium:solute symporter family signatures and have 14 transmembrane domains. It is now believed that SGLT1 is largely responsible for glucose and galactose transport across the intestinal brush border, and that it plays only a minor role, perhaps in the distal tubule, in glucose reabsorption in the kidney. Glucose transport in the proximal tubule of the kidney is mainly attributed to SGLT2, which has a low affinity for sugars and a high selectivity for glucose over galactose. The function and transport activity of other Na^+/glucose cotransporters remain as yet unclear.

BOVINE GLUCOSE TRANSPORTERS

To our knowledge, the full-length cDNA sequences of bovine glucose transporters that have been reported thus far are GLUT1 (GenBank accession #NM_174602), GLUT3 (NM_174603), GLUT4 (NM_174604), GLUT8 (AY208940), GLUT12 (AY514443), SGLT1 (AF508807), SGLT2 (AY208941), and SGLT5 (AY514442). Here we summarize our current knowledge about some of these transporters, which are mostly relevant to glucose transport in the bovine mammary gland.

GLUT1

Bovine GLUT1 (**bGLUT1**), deduced from its full-length cDNA (Boado and Pardridge, 1990), contains 492 AA with a predicted molecular weight of 54 kDa. However, in PAGE, both the in vitro transcription/translation product of the full-length bGLUT1 cDNA (Zhao et al., 2004) and the protein bands detected in bovine tissues by Western blot using an anti-GLUT1 antibody (Boado and Pardridge, 1990; Zhao et al., 1996a) show the molecular weight to be around 40 kDa. The reason for this discrepancy is uncertain, and it is possible that this may be due to some form of posttranslational modification. The deduced bGLUT1 AA sequence is highly conservative and exhibits approximately 96 to 98% sequence identity to the human, rat, and mouse GLUT1. However, there is a unique proline-rich sequence between the putative first 2 transmembrane domains, just close to the predicted N-glycosylation site at Asn^{45} of bovine bGLUT1 (Boado and Pardridge, 1990). The significance of this structure is not known. A search of the current GenBank bovine genomic database indicates that bGLUT1 is a single-copy gene located on chromosome 11. The gene consists of 10 exons as in the human and extends over 28 kb. The bGLUT1 can be glycosylated (Zhao et al., 2004), which has been shown to be critical for the transport activity for GLUT (Asano et al., 1991), and may explain the doublet bands for GLUT1 proteins observed in bovine tissues (Boado and Pardridge, 1990; Zhao et al., 1996a).

The GLUT1 mRNA is ubiquitously expressed in lactating bovine tissues, being most abundant in the mammary gland and kidney and lowest in the omental fat and skeletal muscle, with a single transcript of 2.8 kb (Zhao et al., 1993). It is also expressed in fetal bovine tissues (Hocquette et al., 2006) and the bovine follicle and corpus luteum (Nishimoto et al., 2006). Protein levels of GLUT1 are significantly higher in glycolytic than in oxidative muscles (Duhlmeier et al., 2005). In the brain, bGLUT1 mRNA is specifically expressed only in the blood-brain barrier (Boado and Pardridge, 1990). During postnatal development, GLUT1 protein levels are unchanged in the bovine skeletal muscle, adipose tissue, and brain (Abe et al., 2001). However, GLUT1 expression in adipose tissue is dramatically changed during different stages of lactation, from very low levels in early lactation to relatively strong levels in late and nonlactating stages (Komatsu et al., 2005).

Regulation of GLUT1 expression has been studied extensively in vitro. In general, GLUT1 expression is induced by many growth stimuli to meet the increased energy and biosynthetic demand of dividing cells. For example, in bovine chromaffin cells, GLUT1 mRNA is elevated by glucose deprivation and IGF-I activation (Fladeby et al., 2003). Evidence indicates that the bGLUT1 gene may also be regulated at the translational and posttranslational levels (Boado and Pardridge, 1990; Boado, 2000).

GLUT4

The full-length bovine GLUT4 (**bGLUT4**) cDNA is 2,656 bp and is predicted to encode a protein of 509 AA, with a molecular weight of approximately 55 kDa (Abe et al., 1997). Bovine GLUT4 is 65% identical to bGLUT1 and is highly conserved with human and rodent GLUT4, with a possible N-linked glycosylation site at Asn^{57} located between the putative first 2 transmembrane domains. The GLUT4 protein may be subject to differential glycosylation in bovine adipose tissue, heart, and skeletal muscle (Hocquette et al., 1997). The single-copy bGLUT4 gene is located on chromosome 19 and consists of 11 exons and 10 introns, but extends over only 5.4 kb.

In the bovine, GLUT4 mRNA expression is mainly confined to the insulin-sensitive tissues, such as the skeletal muscle, heart, and adipose tissue, essentially the same as in human tissues (Zhao et al., 1993; Abe et al., 1997), suggesting a role in insulin regulation of glucose uptake in these tissues, as occurs in other species. Interestingly, GLUT4 is also expressed in preimplantation embryos (Navarrete Santos et al., 2000). In fetal bovine perirenal adipose tissue, GLUT4 protein levels markedly increase during fetal development, reach maximal expression at 6 to 8 mo of age, and sharply decrease thereafter, with a simultaneous increase in the GLUT1 protein level (Hocquette et al., 2006). During postnatal development, GLUT4 levels in the skeletal muscle and subcutaneous adipose tissue decrease gradually, and at 12 mo old, GLUT4 levels are only 40% of those seen at birth (Abe et al., 2001). In growing calves, GLUT4 protein levels are higher in omental fat than in subcutaneous adipose tissues (Hocquette et al., 1997). In contrast to GLUT1 expression and rodent GLUT4 expression, bGLUT4 protein levels are significantly higher in oxidative than in glycolytic muscles (Hocquette et al., 1995; Duhlmeier et al., 2005). In addition, GLUT4 mRNA expression in bovine adipose tissue and skeletal muscle does not seem to vary with the stage of lactation (Komatsu et al., 2005).

GLUT4 has been extensively studied in humans and rodents because of its key role in the regulation of glucose homeostasis by insulin. The GLUT4 protein is located in an intracellular compartment without insulin stimulation. However, in response to insulin stimulation, GLUT4 is translocated to the plasma membrane, resulting in increased glucose uptake in muscle and adipose tissues (Watson et al., 2004; Watson and Pes-

sin, 2006). In bovine muscle, the insulin-induced GLUT4 translocation to the plasma membrane is significantly lower than in porcine muscle, which may explain the lower insulin sensitivity in adult ruminants compared with monogastric omnivores (Duhlmeier et al., 2005). It has been speculated that the replacement of Asn^{508} in the C-terminus of human and rodent GLUT4 by His^{508} in bGLUT4 may contribute to the impaired insulin stimulation of translocation (Abe et al., 1997).

In the lactating cow, GLUT4 expression in the skeletal muscle and omental fat is dramatically reduced by administering GH and GH-releasing factor (**GHRF**), consistent with the regulation of nutrient partitioning by these hormones to shift more glucose from these tissues to the mammary gland for the purpose of increased milk synthesis (Zhao et al., 1996b).

GLUT8

The full-length bovine GLUT8 (**bGLUT8**) cDNA is composed of 2,073 bp and encodes for a 478-AA protein with a molecular weight of 51 kDa (Zhao et al., 2004). The deduced bGLUT8 protein is only 26 and 24% identical to bGLUT1 and bGLUT4, respectively, but is 90 and 84% identical to human and mouse GLUT8. The bGLUT8 retains an N-linked glycosylation site on loop 9 and a putative dileucine internalization motif. The glycosylation of bGLUT8 was confirmed by the increased molecular weight of an in vitro transcription/translation product of bGLUT8 cDNA in the presence of canine microsomal membranes (Zhao et al., 2004). The bovine genome contains a single copy of the GLUT8 gene, which is located to chromosome 11 as GLUT1 and consists of 10 exons spanning 9 kb. A 2.1-kb bGLUT8 transcript is predominantly expressed in the testes, as in other species, but is also expressed in all other tissues examined, including the mammary gland, kidney, lung, spleen, intestine epithelia, skeletal muscle, and liver (Zhao et al., 2004).

GLUT12

Bovine GLUT12 (**bGLUT12**) cDNA has recently been cloned. The bGLUT12 gene is located to a single locus on chromosome 9 and consists of 5 exons ranging from 123 to 1,341 bp and 4 introns ranging from 4,011 to 10,797 bp. The gene spans 36 kb (Miller et al., 2005) and is transcribed to a 2,423-bp full-length mature mRNA, predicted to encode a protein of 621 AA with a molecular weight of 67 kDa (Miller et al., 2005). The deduced AA sequence of bGLUT12 is 87 and 82% identical to human and mouse GLUT12 and only 20, 20, and 21% identical to bGLUT1, bGLUT4, and bGLUT8, respectively. The bGLUT12 can also be glycosylated (Miller et al., 2005). The bGLUT12 mRNA transcript is ubiquitously expressed in bovine tissues, being most abundant in the spleen and skeletal muscle, at intermediate levels in the kidney, testes, and mammary gland, and at lower levels in the liver, lungs, and intestine (Miller et al., 2005). This tissue distribution pattern is different from the insulin-sensitive tissue-restricted expression of GLUT12 in the human (Rogers et al., 2002).

SGLT1 and SGLT2

Although both SGLT1 and SGLT2 were cloned more than a decade ago and have been well characterized in human and rodent species, bovine SGLT1 (**bSGLT1**) and SGLT2 (**bSGLT2**) were only recently cloned (Zhao et al., 2005a,b). Bovine SGLT1 is a 664-AA protein with a molecular weight of 73 kDa (Zhao et al., 2005b). The 2,275-bp bSGLT2 mRNA encodes a 673-AA protein with a molecular weight similar to bSGLT1 (Zhao et al., 2005a). Both proteins are 58% identical to each other and contain several characteristically conserved sodium:solute symporter family signatures. The bSGLT1 can be glycosylated, whereas glycosylation of bSGLT2 is unclear because the canine microsomal membranes shift the bSGLT2 protein weight only slightly (Zhao et al., 2005a). The bovine genome contains a single copy of each gene. The bSGLT1 gene is located on chromosome 17 and consists of at least 15 exons extending over at least 47 kb. The bSGLT2 is located on chromosome 25 and consists of 14 exons that span only 9 kb. Expression of SGLT1 mRNA is most abundant in bovine intestinal tissues, at intermediate levels in the bovine kidney, at lower levels in the bovine mammary gland, liver, and lungs, and not detectable in the bovine spleen, skeletal muscle, and testes (Zhao et al., 2005b). Interestingly, SGLT1 mRNA is strongly expressed in the rumen and omasum of lactating cows, suggesting that these tissues may be involved in glucose absorption (Zhao et al., 1998). As in other species, the SGLT2 mRNA is predominantly expressed in the bovine kidney as a 2.3-kb transcript, and is expressed at lower levels in the bovine mammary gland, liver, lung, spleen, intestine, and skeletal muscle as a 3.0-kb transcript (Zhao et al., 2005a). Expression of Na^+/glucose transporters in the mammary gland, liver, lungs, spleen, and skeletal muscle raises questions about their physiological roles in these tissues because the glucose uptake in these tissues is believed to be a facilitative process.

GLUCOSE TRANSPORT AND GLUCOSE TRANSPORTERS IN THE MAMMARY GLAND

Glucose transport into the epithelial cells of the mammary gland is specific, saturable, Na^+-independent, and

inhibitable by cytochalasin-B or phloretin in the guinea pig, rat, mouse, and cow (Amato and Loizzi, 1979; Threadgold et al., 1982; Prosser, 1988; Delaquis et al., 1993; Xiao and Cant, 2003; Xiao et al., 2004). For uptake of 3-*O*-methy-D-glucose in bovine mammary epithelial cells, the K_m is 8.29 m*M* and the V_{max} is 18.2 nmol/min per mg of protein (Xiao and Cant, 2003). In lactating rat mammary acini, the apparent K_m for 2-deoxy-D-glucose is 16 m*M*, and the V_{max} is approximately 56 nmol/min per mg of protein (Threadgold et al., 1982).

The glucose transporters in the mammary gland have primarily been reported in a few studies in the rat. Burnol et al. (1990) and Camps et al. (1994) reported that GLUT1 and GLUT4 are present in the rat mammary gland before conception. However, the expression of GLUT4 decreases progressively during pregnancy and becomes undetectable during lactation, whereas the levels of GLUT1 increase during pregnancy and peak during lactation. The pattern of GLUT1 and GLUT4 expression during the reproductive cycle reflects differences in the cellular composition of the mammary gland. Adipocytes, which express both GLUT4 and GLUT1, predominate before pregnancy, whereas epithelial cells, which express GLUT1 but not GLUT4, proliferate and become the predominant cell type during pregnancy and lactation. More recently, GLUT12 has been found in the rat mammary gland (Macheda et al., 2003). Immunofluorescence and immunoelectron studies have shown that GLUT1 is localized to the basolateral membrane and Golgi apparatus of the lactating mammary epithelium (Takata et al., 1997; Nemeth et al., 2000; Macheda et al., 2003), whereas GLUT12 targets the apical membrane (Macheda et al., 2003). Madon et al. (1990) have used quantitative Western blotting and cytochalasin-B binding studies to demonstrate that GLUT1 represents the major glucose transporter species in plasma membranes and about half of the glucose transporters in the Golgi membranes of lactating rat mammary epithelial cells. The presence of glucose transporters on Golgi vesicle membranes raises new questions with respect to the presence of pores in these membranes. Because only about half of the sites on Golgi vesicle membranes could be accounted for by GLUT1, it may indicate the existence of other glucose transporter species in the Golgi membrane.

Interestingly, GLUT2 protein is not expressed in the bovine and rat mammary gland (Burnol et al., 1990; Madon et al., 1990; Zhao et al., 1993) but is present in human breast tissue (Brown and Wahl, 1993). Neither GLUT3 nor GLUT5 is expressed in human or rat mammary tissues (Brown and Wahl, 1993; Camps et al., 1994). Surprisingly, there is also evidence for the presence of Na^+/glucose cotransporters in the lactating mammary gland (Zhao et al., 1999; Shennan and Peaker, 2000).

Glucose Transporters in the Bovine Mammary Gland

In the early 1990s, when only GLUT1 to GLUT5 had been cloned, human cDNA were used in Northern blotting analyses of bovine tissues (Zhao et al., 1993), and it was demonstrated that only GLUT1 mRNA was found at high levels in the mammary gland of lactating cows. The GLUT3, GLUT4, and GLUT5 mRNA were also detected, but at very low levels in 10 μg of poly (A^+) RNA used. Later, the expression of the Na^+/glucose cotransporter SGLT1 in the lactating bovine mammary gland was also detected (Zhao et al., 1998 and 1999). High levels of GLUT1 protein were also detected in both the lactating and involuted mammary gland, whereas GLUT4 protein expression was not detected (Zhao et al., 1996a). In addition, an anti-GLUT1 antibody strongly stained the single layer of epithelial cells of mammary alveoli (Zhao et al., 1996a).

Since then, new glucose transporters have been identified. To determine which of these novel glucose transporter isoforms (GLUT6 to GLUT12 and SGLT2 to SGLT3) were expressed in the bovine mammary gland, the GenBank databases were initially BLAST-searched using the human sequences of these isoforms to find expressed sequence tags (**EST**) clones derived from bovine mammary tissue. This approach allowed the identification of EST clones for GLUT8 and SGLT2 in the USDA Meat Animal Research Center (ARS) cDNA library of bovine mammary tissues (Zhao et al., 2004). The full-length cDNA of these 2 transporters were subsequently cloned and their expression confirmed in lactating bovine mammary tissue (Zhao et al., 2004; Zhao et al., 2005a).

In addition, although EST clones for GLUT12 were not found in the bovine mammary gland cDNA library, GLUT12 has been found to be highly expressed in rat mammary tissues and in the human breast cancer cell line MCF-7 (Rogers et al., 2002; Macheda et al., 2003). To examine expression of GLUT12 in the bovine mammary gland, rapid amplification of cDNA ends in lactating mammary tissue was carried out using specific bGLUT12 primers designed from GenBank EST sequences, and rapid amplification of cDNA end products were successfully obtained, confirming GLUT12 expression (Miller et al., 2005). The full-length bGLUT12 cDNA was subsequently cloned from the lactating bovine mammary gland (Miller et al., 2005).

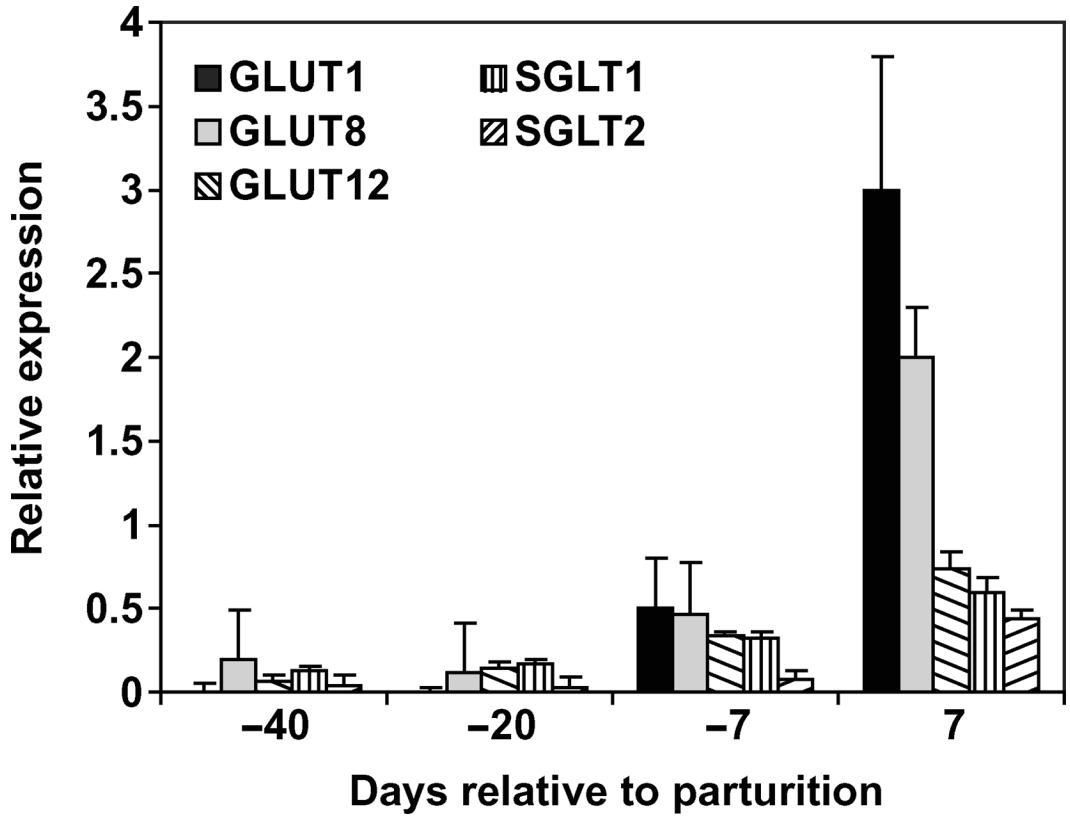

Figure 1. Quantitative reverse transcription PCR analysis of GLUT1, GLUT8, GLUT12, SGLT1, and SGLT2 mRNA expression in the bovine mammary gland at –40, –20, –7, and 7 d relative to parturition. The mammary tissues were taken by biopsy from 12 cows at each stage. The mRNA levels of each transporter were normalized to β-actin levels. Data are taken from Zhao et al. (2004, 2005a,b) and unpublished data (F.-Q. Zhao, E. H. Wall, and T. B. McFadden).

REGULATION OF GLUCOSE TRANSPORTER GENE EXPRESSION IN THE BOVINE MAMMARY GLAND

Developmental Regulation

Glucose uptake in the mammary gland is increased dramatically during lactation. To study the changes in GLUT1, GLUT8, GLUT12, SGLT1, and SGLT2 expression in the bovine mammary gland at the onset of lactation, quantitative, real-time reverse transcription PCR was used to analyze their mRNA levels in mammary tissues taken by biopsy from 12 cows at d –40, –20, –7, and +7 relative to calving. Expression of all transporters examined has been shown to be developmentally regulated during pregnancy and lactation, as evident from the marked increase in expression seen during the onset of lactation (Figure 1). The mRNA levels of GLUT1, GLUT8, GLUT12, SGLT1, and SGLT2 increased a few hundred-, 10-, 10-, 4-, and 10-fold, respectively, from d –40 to 7. The patterns and relative expression levels among the different transporters are highly correlated both with each other and also with the beginning of milk synthesis. Levels of GLUT1 are barely detectable at d –40 and –20; however, in anticipation of the increased lactose synthesis requirement occurring with the onset of lactation, GLUT1 expression is greatly increased at d –7 and is further increased approximately 6-fold at d 7 when lactation is established. The expression pattern of GLUT8 mRNA is comparable to that of GLUT1; however GLUT8 levels are detectable at higher levels than GLUT1 at d –40 and –20. In the same manner as GLUT1, the expression level is increased at d –7 and is further increased approximately 4-fold with lactation d 7. These expression patterns are consistent with those detected by microarray analysis in the mouse mammary gland (Zhao et al., 2004). GLUT1 and GLUT8 have the highest levels of expression of the 5 transporters examined in Figure 1. Transporters GLUT12 and SGLT1 follow a pattern of expression similar to GLUT1 and GLUT8; however, their mRNA levels are considerably less at d 7. The expression pattern of SGLT2 is slightly different from those of the other transporters examined, with a smaller in-

crease in expression at d –7. However, expression also increases considerably with the onset of lactation. The increase in expression during the onset of lactation indicates that all of these transporters may play specific roles to support milk synthesis. Consistent with these observations, Komatsu et al. (2005) recently reported that GLUT1 mRNA and protein are strongly expressed in the bovine mammary gland at lactation but that they are barely detectable in dry cows. However, in the same study, GLUT1 mRNA and protein abundance in the mammary gland were found unchanged at peak and late lactation. These results are contrary to our previous report that GLUT1 protein levels are significantly lower in the mammary gland at 181 d of lactation than at 118 d, whereas GLUT1 mRNA levels remain unchanged (Zhao et al., 1996a). In addition, GLUT1 protein was detected with different molecular weights in lactating and dry stages, implying that GLUT1 may be subjected to differential posttranslational modifications at different stages and may then be subjected to a change in its transport kinetics (Zhao et al., 1996a).

Hormonal Regulation

A study was performed to investigate the effect of administration of GH and GHRF. Exogenous GH had no effect on either GLUT1 mRNA or protein, although milk yield increased 17%. In contrast, GHRF increased milk yield (by 14%) and GLUT1 mRNA levels (Zhao et al., 1996b). Both bovine GH and bovine GHRF dramatically decreased GLUT4 expression in peripheral adipose and muscle tissues, consistent with regulation of nutrient partitioning by these hormones to shift more glucose from these tissues to the mammary gland to support milk synthesis (Zhao et al., 1996b,c). In addition, leptin changed neither glucose uptake nor GLUT1 mRNA expression in a bovine mammary gland explant culture study (Accorsi et al., 2005).

The developmental regulation of the glucose transporters prior to and after the beginning of lactation indicates that their expression and function are likely to be regulated by lactogenic hormones around the time of lactation. Intracellular GLUT-1 concentrations in mouse mammary epithelial cells have been shown to increase approximately 15-fold in response to prolactin and hydrocortisone (Haney, 2001). However, the regulation of glucose transporter expression in the bovine mammary gland by these hormones remains to be elucidated.

CONCLUSIONS AND PERSPECTIVE

Glucose uptake in the bovine mammary gland is of major importance for successful lactation in the dairy cow. Until recently, the majority of research carried out has been on GLUT1 in the mammary gland and GLUT4 in the adipose tissue. However, it is becoming more obvious from the identification of additional glucose transporters in the mammary gland, especially GLUT8, GLUT12, and SGLT1, that glucose uptake from the circulation into the mammary epithelial cell is a more complicated system that involves the coordination of a number of proteins. The expression of these transporters is both developmentally and hormonally regulated. Future studies are required to localize each of these transporters cellularly and subcellularly in the bovine mammary gland because their localizations may determine their physiological function. Of similar importance is determination of the transport kinetics of each transporter in the mammary gland. Adapting advanced research approaches, such as using specific gene-knockout animal models and siRNA approaches, should also provide useful information in understanding the functions of each transporter during milk synthesis. In addition, future studies to investigate the regulation of gene expression and posttranslational modifications of each transporter by lactogenic hormones will be critical. A thorough understanding of the molecular mechanisms of glucose uptake and its regulation in the mammary gland is a prerequisite for enhancing glucose utilization in the mammary gland and ultimately for improving dairy productivity and efficiency.

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