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Relationship Between Glucose Transport and Metabolism in Isolated Bovine Mammary Epithelial Cells

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ABSTRACT

Glucose transport by isolated bovine mammary epithelial cells involves translocation across the cell membrane into a compartment that exchanges slowly with the bulk cytosol. The significance to glucose metabolism of this compartmentalization was examined by generation, modeling, and analysis of transport and metabolism data. Net uptake of 5 m*M* 3-O-methyl-D-glucose by isolated bovine mammary epithelial cells was measured at 37°C. Time-course curves were better fitted by a double exponential equation than a single exponential equation and were subjected to compartmental analysis to obtain glucose transport model parameters. Lactose synthesis and glucose oxidation rates and cellular concentrations of intermediary metabolites, glucose-6-phosphate and glucose-1-phosphate, were measured at varied media glucose concentrations. A model that integrates both glucose transport and metabolism underpredicted the rates of lactose synthesis and glucose oxidation by a factor of 3. To account for the observed glucose use rates, glucose must be available for phosphorylation once translocated across the cell membrane (intermediate compartmentalization of translocated glucose does not exclude access to hexokinase). Metabolic control analysis indicated that, at physiological glucose concentrations, phosphorylation by hexokinase exerts 80% of the control of glucose metabolism to lactose and CO_2, and transport exerts the remaining 20%. (**Key words:** milk synthesis, mathematical modeling, glucose transport, metabolism)

Abbreviation key: 3-OMG = 3-O-methyl-D-glucose, **CCB** = cytochalasin B, **DMEM** = Dulbecco's Modified Eagle's medium, **G1P** = glucose-1-phosphate, **G6P** = glucose-6-phosphate, **GLUT** = glucose transporter, **HK** = hexokinase, **MSPE** = mean square prediction error, **rMSPE** = square root of the mean square prediction error.

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INTRODUCTION

A continuous supply of glucose from the blood circulating in the mammary gland is essential to maintain lactation. Up to 85% of whole-body glucose turnover is directed to the mammary glands for milk synthesis (Bickerstaffe and Annison, 1974). Synthesis of the disaccharide lactose in the Golgi apparatus represents the major fate of glucose metabolism in the mammary epithelial cells, and glucose oxidation facilitates the synthesis of other milk components such as fatty acids (Mepham, 1987). Through osmosis, lactose is the major determinant of milk volume (Holt, 1983). Therefore, its synthesis dictates the milk yield and percentage composition of other components in the milk.

In the lactating mammary epithelial cell, based on the low intracellular concentration of glucose and the similarity between rates of glucose consumption and uptake, it has been suggested that glucose transport from plasma into the cell is rate-limiting to lactose synthesis (Wilde and Kuhn, 1981; Threadgold et al., 1982; Threadgold and Kuhn, 1984). Evidence to the contrary has accumulated. Infusion of glucose into an artery proximal to the mammary glands of lactating cows increased mammary glucose uptake by 40%, but mammary blood flow declined and lactose synthesis was elevated by only 6% (Cant et al., 2002). Duodenal infusion of 1500 or 2400 g/d glucose had little effect on mammary lactose synthesis although intracellular glucose concentration was elevated (Hurtaud et al., 1998; Rigout et al., 2002). Metabolic control analysis (Fell, 1992) provides a methodology to quantitatively assess the degree of control individual enzymes or transporters exert over the flux through a metabolic pathway. Recent applications of control analysis to glucose metabolism in *Trypanosoma brucei* (Bakker et al., 1999) suggested that transport exerted 30 to 50% control at 5 m*M* glucose. In human skeletal muscle cells, phosphorylation by hexokinase (**HK**) exerted 15 to 20% control over glucose metabolic rates (Perriott et al., 2001). Similarly, although glucose supply was the primary factor that regulated glycogen accumulation in skeletal muscle at normal state, glycogen synthase activity also contributed to the control of glycogen accumulation in the fasting state (Ren et al., 1993; Fisher et al., 2002). The relative

control over glucose metabolism by its transport and phosphorylation has not been determined in bovine mammary epithelial cells.

An appropriate description of glucose transport kinetics, particularly of its reversibility, must be considered for control analysis. Lactating mammary epithelial cells use glucose transporter (**GLUT**) to move glucose across the basolateral membrane (Zhao et al., 1996). Initial glucose entry rates into mammary epithelial cells measured over a short time interval exhibit Michaelis-Menten saturation kinetics relative to glucose concentration (Threadgold et al., 1982; Xiao and Cant, 2003). However, the intracellular concentration of glucose influences the apparent K_m and V_{max} of entry (Xiao and Cant, 2003), as shown for human erythrocytes (Wheeler and Whelan, 1988; Cloherty et al., 1996). It has been suggested that the effect of intracellular glucose is an artifact of its compartmentalization (Carruthers, 1991; Naftalin and Rist, 1991; Xiao et al., 2004). A compartmental model of symmetric, carrier-mediated translocation of glucose across the plasma membrane into an occlusion space that also exchanges by diffusion with the bulk cytosol can reproduce the anomalous kinetic behavior of initial entry rates in human erythrocytes (Heard et al., 2000) and bovine mammary epithelial cells (Xiao et al., 2004). It has been proposed that the occlusion space is a cage formed by cytoplasmic domains of GLUT1 molecules organized in a homotetramer under the influence of cytosolic ATP (Heard et al., 2000). Whether glucose in the occluded space is available for phosphorylation by HK and subsequent metabolism is unknown. The objectives of this study were to simultaneously measure kinetics of glucose transport and oxidation and lactose synthesis in bovine mammary epithelial cells and, by integrating these separate measures together in simulation models, to estimate the degree of control of glucose transport over glucose metabolism using the equations of metabolic control analysis.

MATERIALS AND METHODS

Materials

All chemicals and enzymes were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise stated. 3-O-Methylglucose (**3-OMG;** 167 GBq/mmol; 99.8%) and D-[U-^{14}C]glucose (12.0 GBq/mmol, 98.7%) were from Amersham Life Science (Buckinghamshire, UK). The NAD(P)H:oxidoreductase (EC 1.6.8.1) and glucose-6-phosphate dehydrogenase (EC 1.1.1.49) were supplied by Roche Diagnostics (Laval, Quebec, Canada). Hank's Balanced Salt Solution and Dulbecco's Modified Eagle's medium (**DMEM**) base were from GibcoBRL (Life Technologies, Burlington, Ontario, Canada).

Cell Preparation

On 3 separate occasions, epithelial cells were prepared as previously described (Xiao and Cant, 2003) from mammary tissue obtained at slaughter from a lactating Holstein dairy cow producing milk at 15 kg/d. Minced tissue was digested with 0.1% (wt/vol) collagenase in Hank's Balanced Salt Solution for 75 min. Cells were collected via centrifugation and washed 3 times with ice-cold PBS. Cells were then resuspended in glucose-deficient DMEM base at a protein concentration of 2 to 4 mg/mL and stored on ice for 3-OMG accumulation and glucose metabolism experiments. Both sets of experiments were completed within 6 h of tissue collection. Cell protein was analyzed by the BioRad assay kit using BSA as the standard.

Time Course of Accumulation of 3-OMG by Isolated Cells

Net uptake of 3-OMG into cells from the 3 cows was measured at 37°C by incubating 100 μL of cell suspension with 100 μL of DMEM base medium containing 10 m*M* 3-O-methyl-D-[1-^{3}H]glucose (10 μCi/mL) for various periods between 15 s and 30 min. Where cytochalasin B (**CCB**) effects were examined, CCB was included in the incubation media to give a final concentration of 0.3 μM. At the end of each incubation interval, uptake was terminated by addition of 3 mL of ice-cold PBS (pH 7.4) containing 20 μM CCB. Cells were then loaded onto Whatman GF/C filters (Fisher Scientific, Whitby, Ontario, Canada) premoistened with PBS, and mounted on a Millipore vacuum filtration unit. Filters were immediately washed with 30 mL of ice-cold PBS. Filters were transferred into 20-mL scintillation vials, soaked with 0.5 mL of water for 30 min. Ten milliliters of scintillation fluid (ICN Pharmaceuticals, Inc., Aurora, OH) was then added to each vial. Vials were counted in a Beckman 6000 scintillation counter. Blanks (time zero uptake) were prepared by addition of stopping solution before addition of incubation media containing 3-OMG and radiolabel, followed by immediate wash. Nonspecific binding of radiolabel to filters was monitored by filtration and washing of incubation media alone. Uptakes were measured in triplicate for each time point and from each cow.

Glucose Metabolism

All incubations were carried out at 37°C for 60 min. After prewarming at room temperature for 30 min, 200-μL cell suspensions were mixed with 400 μL of DMEM base supplemented with amino acids and different concentrations of glucose to give final glucose concentrations of 0.5 to 10 m*M*. Where applicable, CCB was in-