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Several Amino Acids and Carnitine Transport Activities of the Epithelial Cells of Bovine Mammary Gland

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Abstract: We investigated several amino acids and carnitine transport activities of bovine mammary gland epithelial cells. Gly, Ala, Gln, Glu, Arg, Leu, cystine and carnitine transport activities at 1 μmol/L substrate concentration were 24.0 ± 3.97, 90.9 ± 13.4, 32.5 ± 9.0, 14.2 ± 5.1, 48.9 ± 11.4, 48.8 ± 5.1, 22.7 ± 6.8 and 2.56 ± 0.96 nmol/mg protein/min, respectively. Na-dependency of transport was observed in Gly, Ala and Gln, but not in Arg, Leu and carnitine. Glu and Cys transport activity without Na condition were reduced to 36%, 63% in Na-free condition, respectively. Carnitine transport activities were low but detectable with or without Na condition. There was no correlation between amino acid transport activities and their concentrations in milk. The data clarified in this paper will be basic data for metabolic analysis of bovine mammary gland.
Key words: Cow, Mammary gland, Transport activity

Introduction

Many amino acid transport systems have been distinguished based on differences in their substrate-selectivity, ion-dependence, pH sensitivity, kinetics and regulatory properties by using membrane vesicle preparations or cultured cells[1-3]. For example, the amino acid transport system A, N, B^{0+} and G were Na-dependent, and system L, b^{0+} and y^+ were Na-independent. The mammary gland has a high demand for amino acids requiring their uptake for cell proliferation during pregnancy and for milk protein synthesis during lactation. Nutrient provision to lactating mammary gland includes two factors: blood nutrient concentration and cellular uptake. As the Na content in blood was high compared to intracellular Na owing to Na- K pump, the steep Na-gradient made the Na-dependent transporters active in these cells.

Carnitine (3-hydroxy-4-*N*-trimethylaminobutyric acid) is a small, water-soluble molecule that has important physiological roles, including involvement in the β-oxidation of fatty acids. It is synthesized endogenously from lysine (carbon backbone) and methionine (methl group donor) in the liver and/or kidney, and animals being nursed depend on milk for its supply. Notably, bovine milk contains a 0.1 mmol/L order concentration which is the same level as that of amino acid[4]. While amino acids and carnitine in bovine milk are considerably important for calves nutrition, little information is available on the transport activities and their Na-dependency of the

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mammary gland epithelial cells. Therefore, we investigated the transport activity of several amino acids and carnitine using bovine mammary gland epithelial cells to compare the transport activities and their concentrations in bovine milk.

Materials and Methods

BMGE+H cell line derived from epithelial cells of bovine mammary gland was maintained as described[5, 6]. In brief, mammary gland epithelial cells were obtained from lactating cow using the dissociation procedure[7]. Cell cultures of passage 10 were propagated in Dulbecco's minimal essential medium supplemented with 20% fetal calf serum and hormones (insulin, hydrocortisone and prolactin; 1 μg/ml each). The cells became confluent after 3-4 days and the sub-cultivation ratio was 1:5. The cells were maintained for years and used in this study.

Radioactive (^{3}H- or ^{14}C-) chemicals were purchased from American Radiolabeled Chemicals (St. Louis, MO, USA). Na-independent amino acid transport activities were measured as described previously[8]. In brief, the cells were plated 5×10^{5} cells/6-well plate 24 h before the experiment. The cells were washed by buffer containing 130 (mmol/L) NaCl, 5 KCl, 2 $MgCl_2$, 10 glucose, 15 Tris/MOPS and 0.1% BSA. All buffers containing Na were replaced with N-methyl-D-glucamine to observe the Na-dependency. After washing the cells, the medium containing 1 μmol/L of each substrate (Gly, Ala, Gln, Glu, Arg, Leu, Cys and carnitine) with a radioisotope was added and incubated at 37°C for 4 min. Uptake was terminated by washing with 2 ml of ice-cold phosphate-buffered saline three times. After solubilizing the cells with 1 ml of 1% SDS, radioactivity of 0.8 ml cell lysis solution was measured with a liquid scintillation counter, and the protein content of aliquot was determined by the Micro BCA Protein Assay kit (Thermo Scientific, Rockford, IL, USA).

Results and Discussion

The amino acids and carnitine transport activity of bovine mammary gland epithelial cells with or without a Na condition are indicated in Figure 1. Gly, Ala, Gln, Glu, Arg, Leu, cystine and carnitine activities with Na condition were 24.0 ± 4.0, 90.9 ± 13.4, 32.5 ± 9.0, 14.2 ± 5.1, 48.9 ± 11.4, 48.8 ± 5.1, 22.7 ± 6.8 and 2.6 ± 1.0 nmol/mg protein min, respectively. The order of transport potency was Ala > Arg, Leu > Gly, Gln > Cys > Glu > carnitine. On the other hand, the order of amino acid concentration in milk from the greatest downward was Glu (0.18 mmol/L) > Gln (0.06), Gly (0.05), Ala (0.04) > Arg (0.02), Leu (0.01)[9]. Therefore, there was no correlation between activities of amino acid transport in epithelial cells of bovine mammary gland and amino acid concentrations in bovine milk. This discrepancy may be in part due to biosynthesis of each nutrient in epithelial cells. Na-dependency was observed in Gly, Ala and Gln, but was not observed in Arg, Leu and carnitine. Glu and Cys transport activity without Na condition were reduced to 36% and 63%, respectively. The ambiguity of Na-dependency in Glu and Cys transports could be possibly due to the system x^{-}_{c}, Na-independent cystine/glutamate exchanger. Predicted amino acid transport systems were indicated (Fig. 1).

Carnitine transport activities were low but detectable both with or without Na condition. Carnitine has important physiological roles, including involvement in the β-oxidation of fatty acids by facilitating the transport of long-chain fatty acids across the mitochondrial inner membrane as their acylcarnitine esters, and modulation of intracellular CoA homeostasis[10, 11]. Carnitine deficiency causes severe pathological symptoms such as cardiomyopathy and muscle weakness in human.[12, 13] In addition, carnitine infusion effectively decreased liver lipid accumulation in cow as the result of a greater capacity for hepatic fatty acid oxidation (Carlson et al. 2006), and carnitine supplementation improved glucose status and diminished the risk of developing metabolic disorders during early lactation[14, 15]. It was reported that there were several carnitine transporters[16, 17]. As the carnitine transporter in bovine mammary gland was not Na-dependent (Fig. 1), Solute carrier family 22A16 (SLC22A16), Na- independent organic cation transporter, is the candidate of this carnitine transporter in mammary

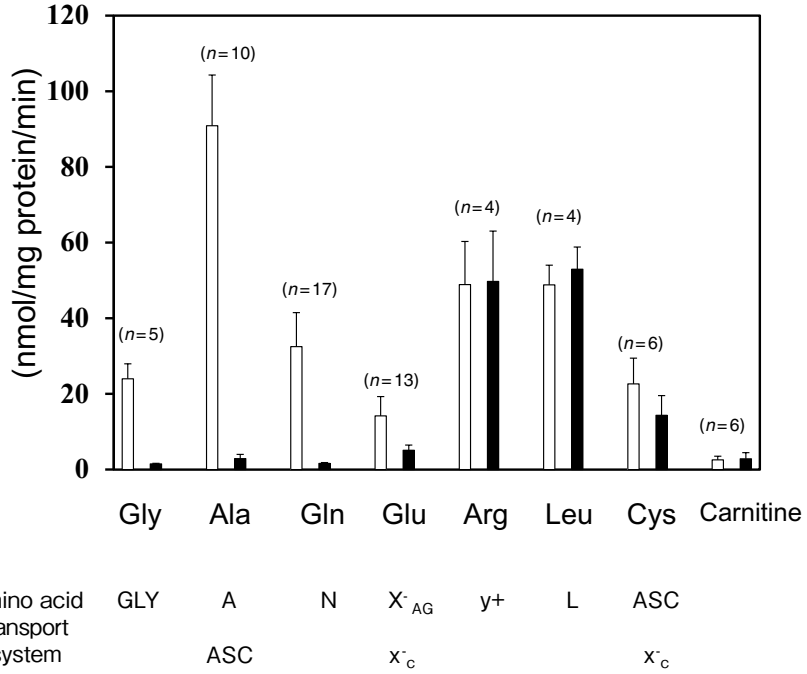

Fig. 1 Amino acid transport activity of epithelial cells of bovine mammary gland with (open column) or without (closed column) Na condition. Values represent the means and SE of separate experiments. "n" above the column is the number of 6-well plates examined (upper). Predicted amino acid transport systems were indicated (bottom).

gland[16].

In this study, we investigated several amino acids and carnitine transport activity and their Na-dependency. It was revealed that there was no correlation between activities of amino acid transport in epithelial cells of bovine mammary gland and amino acid concentrations in bovine milk. To resolve the discrepancy between them, enzyme activities of biosynthesis of each nutrient in cells have to be investigated. The data obtained in this study will contribute to the better understanding of the physiology of the bovine mammary gland.

Compliance with ethical standards

Conflict of interest The authors certify that there is no conflict of interest with any researcher or organization regarding the material used in this manuscript. All authors are aware of the manuscript, which involves the animal.

Committee of ethics and animal welfare All experiments were performed according to the guidelines of the Laboratory Animal Care Committee of Azabu University, and were in compliance with the Fundamental Guideline for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions.

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