Lysine uptake by mammary gland tissue from lactating sows

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ABSTRACT: Kinetic properties and substrate specificity of the lysine transport system in porcine mammary gland were studied using mammary tissue explants from nine lactating sows. Sodium dependence of lysine uptake was determined by replacing sodium in the medium with choline. Kinetic parameters of lysine uptake were determined using lysine concentrations from 5 \( \mu \)M to 5.12 mM. Competition of lysine uptake by other amino acids was determined using the cationic amino acids, arginine and ornithine, and using other essential amino acids. Transport of lysine was time-dependent and was unaffected by replacing sodium with choline. Lysine uptake occurred by a transport mechanism with a \( K_m \) of approximately 1.4 mM and a \( V_{max} \) of 7.9 mmol·kg cell water\(^{-1}\)·30 min\(^{-1}\). Lysine uptake was inhibited by arginine and ornithine and by high concentrations of L-alanine, L-methionine, L-leucine, cycloleucine, and D-lysine, but not by 2-(methylamino)-isobutyric acid. This transport mechanism is the primary system responsible for uptake of cationic amino acids in lactating sow mammary tissue. The relatively high \( K_m \), compared with physiological blood concentrations of lysine, indicates that the kinetic properties of the lysine transport system should not be limiting to milk protein synthesis. Transmembrane transport of lysine by lactating sow mammary tissue should be a direct function of plasma concentrations. However, interactions of other amino acids with the uptake system may affect lysine uptake.

Key Words: Sows, Lactation, Mammary Glands, Amino Acids, Lysine, Transport

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Introduction

Milk production is one of the most important factors limiting neonatal pig growth and pig production (Boyd and Kensinger, 1998). Amino acids requirements of lactating sows are of primary concern for producing sufficient milk to meet the needs of rapidly growing litters (Boyd and Kensinger, 1998). Lactating sows have a high requirement for lysine (NRC, 1998). Supplementation of diets with synthetic lysine may result in deficiencies of other essential amino acids if those amino acids are not also increased in the diet (Richert et al., 1996, 1997). These types of studies of nutrient requirements of lactating sows illustrate the need for a greater understanding of nutrient uptake and utilization at the mammary tissue level. Understanding lysine metabolism by mammary gland is of particular importance in understanding the relationships between mammary amino acid metabolism and synthesis of milk.

Amino acid utilization by mammary tissue is affected by blood concentrations of amino acids, mechanisms of amino acid uptake into mammary cells, and intracellular metabolism of amino acids. The rat mammary lysine transport system (Shennan et al., 1994; Calvert and Shennan, 1996) interacts with a range of amino acids, particularly arginine and neutral amino acids, some of which are extracted by sow mammary gland in excess of requirements for milk production (Linzell et al., 1969; Trottier et al., 1997). Interactions between cationic and neutral amino acids can have physiological consequences when purified amino acids are used to supplement diets (Richert et al., 1996, 1997). The nature of the cationic amino acid transport system in sows is not known. The objective of this study was to characterize the kinetic properties and substrate specificity of the lysine transporter system in lactating sow mammary tissue.

Materials and Methods

Animals. Mammary tissue was collected from five multiparous (one purebred Duroc and four Camborough-15 × line 326, Pig Improvement Company, Franklin, KY) and four primiparous (Camborough-15 × line 326, Pig Improvement Company) sows. Litters were weaned (7.9 ± .8 pigs/litter) and sows slaughtered at
18.7 ± .9 d of lactation. Litters were removed approximately 1 h prior to slaughter of the sow. Sows were obtained from the University of Illinois Swine Research Center herd and moved to the University of Illinois Meat Science Laboratory abattoir for slaughter. Sows were electrically stunned and killed by exsanguination. Tissue was returned to the Animal Sciences Laboratory for the culture experiments. The animal care protocol (#A8R134) was approved by the Laboratory Animal Care Committee of the University of Illinois at Urbana-Champaign.

Amino Acid Uptake by Explants. Tissue was excised from the center portion of three or four lactating glands from each sow. Tissue was minced in basal medium using curved scissors until tissue pieces were approximately .5 mm in diameter. Obvious pieces of connective tissue and large pieces of tissue were removed. Basal incubation medium contained 5 mM KCl, 2 mM CaCl₂·2H₂O, 1 mM MgSO₄, 135 mM NaCl, 10 mM glucose, and 10 mM TRIS-BES, pH 7.4 (Shennan et al., 1994). Minced tissue from each gland was pooled and washed three times in basal medium. Time from tissue harvest to beginning of incubations was approximately 1 h.

To determine the time course of lysine uptake, explants were incubated in basal medium containing .3 mM or 1 mM of lysine, plus radiolabeled tracer for periods up to 120 min. Concentrative uptake of an amino acid occurs when the intracellular concentration of the amino acid exceeds the extracellular concentration. Concentrative uptake of lysine occurred between 20 and 30 min after addition of tracer lysine to the culture medium (Figure 1).

For determining sodium independence, NaCl in the basal medium was replaced with 135 mM choline chloride. Competition of lysine uptake by arginine and ornithine included the competitor amino acids in a range from .32 mM to 40.96 mM. For amino acid uptake studies characterizing competition among amino acids, lysine was included in the medium at 20 μM (plus radiolabeled tracer lysine), and the competitor amino acids were included at 20.48 mM. Each uptake determination was replicated with a minimum of three explant cultures. Amino acids, choline chloride, Tris, and BES were purchased from Sigma Chemical Co. (St. Louis, MO). All solutions were gassed with oxygen (100%) for 5 min.

Rinsed tissue pieces (20 to 40 mg) were briefly placed on blotting paper to remove excess fluid and then placed in a glass, screw-capped, 20-mL scintillation vial, and basal medium (2 mL) containing amino acids to be studied was added. Vials were gassed for 15 s with oxygen (100%) and placed in a shaking water bath (Dubhoff Incubator, Precision Scientific, Winchester, VA) at 37°C for 5 min. After the 30-min preincubation, L-[4,5-3H]-lysine monohydrochloride (93 Ci/mmol, Amersham Life Science, Arlington Heights, IL) was added directly to the vials to a final concentration of 1 μCi/mL, and the vials were incubated for a further 30 min. After incubation, explants were lightly blotted and weighed, and macromolecules were precipitated with trichloroacetic acid. Soluble radiolabeled, representing free amino acid taken up by the tissue, was quantified and uptake was calculated.

Intracellular concentration of amino acid was estimated using the measures of radioactivity in the medium and in the trichloroacetic acid extract of explants after culture. Extracellular space in the explant tissue was estimated by incubating a set of explants with [U-14C]-sucrose (612 mCi/mmol, Amersham Life Science) at a final concentration of .5 μCi/mL. Sucrose is impermeable to the cells and will only equilibrate in the extracellular fluid. Extracellular fluid content of mammary explants averaged 58.9 ± 1.7% of total explant fluid (mean ± SE; n = 8 sows). This agrees with observations in lactating mammary tissue from rats and cows (Baumrucker, 1984; Shennan et al., 1994).

Calculation of Uptake and Statistical Analyses. Uptake of lysine was calculated as described previously (Shennan and McNeillie, 1994). Kinetic parameters (Km and Vmax) were determined using nonlinear regression analysis. Statistical analysis was performed using Student’s t-test, and differences were considered significant when P < .05.

Results and Discussion

Amino acid transport systems in mammary tissue concentrate amino acids in the epithelial cells with respect to plasma or milk amino acid concentrations and
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Figure 2. Sodium dependence of lysine uptake in lactating porcine mammary tissue explants. Uptake was measured with 20 μM lysine in the presence of Na⁺-containing medium or medium in which NaCl was replaced with choline chloride. Values are mean ± SE; n = 6 sows. Comparison of media containing NaCl vs choline chloride was not significant (P > .05).

have a requirement for input of energy. In many amino acid uptake systems, the transmembrane Na⁺-gradient drives the amino acid uptake, and these systems are considered Na⁺-dependent (Shennan et al., 1997; Shennan, 1998). In contrast, the classic y⁺ system for transport of cationic amino acids is Na⁺-independent (Shennan et al., 1997; Shennan, 1998). Uptake of cationic amino acids by mammary tissue of bovines and rats is Na⁺-independent (Baumrucker, 1984; Shennan et al., 1994). In the present study, uptake of lysine by lactating sow mammary tissue was not significantly affected (P = .12) by the presence or absence of sodium in the medium (Figure 2). Therefore, the concentrative uptake of lysine is probably governed by the electrical membrane potential.

Kinetic parameters of lysine uptake by lactating sow mammary tissue were determined by incubating explants in medium containing lysine concentrations ranging from 5 μM to 5.12 mM (Figure 3). Lysine uptake could be described by a single saturable curve with respect to the medium lysine concentration and with a Kₘ and Vₘₐₓ of 1.38 mM and 7.9 mmol·kg cell water⁻¹·30 min⁻¹, respectively. We are aware, however, that the kinetic data could also be described by more complex models. With a Kₘ of nearly 1.4 mM, the lysine transport system should not become saturated under physiological concentrations of blood lysine. Blood lysine concentrations range widely depending on dietary protein and lysine content; serum or plasma lysine concentrations in lactating sows of < 75 to > 600 μM have been reported (Linzell et al., 1969; Spencer et al., 1969; Lewis and Speer, 1973; Chen et al., 1978; Richert et al., 1996, 1997; Trottier et al., 1997).

Uptake of lysine by lactating sow mammary tissue was strongly inhibited by other basic amino acids, namely arginine and ornithine (Figure 4). When cultured in the presence of physiological concentrations of arginine (320 μM) or ornithine (320 μM), uptake of lysine (20 μM) was inhibited 33 and 60%, respectively. Mammary gland has a high requirement for arginine, which is one of several amino acids taken up by mam-

Figure 3. Effect of medium amino acid concentration on lysine uptake by lactating porcine mammary tissue explants. The Kₘ and Vₘₐₓ were estimated as 1.38 mM and 7.9 mmol·kg cell water⁻¹·30 min⁻¹, respectively. Values are mean ± SE; n = 6 sows.

Figure 4. Competition of lysine uptake in lactating porcine mammary tissue explants by arginine and ornithine. Uptake was measured with 20 μM lysine in medium plus arginine (closed circles) or ornithine (open circles). Values are mean ± SE; n = 6 sows.
was partially inhibited by D-lysine, indicating that the cationic amino acids and Na\(^+\) specificity but are Na\(^+\)-dependent, or via systems b\(^{0+}\) or y\(^{\prime}\)L, which have broad substrate specificity but are Na\(^+\)-independent (Devés and Boyd, 1998). Uptake of cationic amino acids in bovine and rat mammary tissues are Na\(^+\)-independent, but they have a broad range of substrate specificity (Baumrucker, 1984; Shennan et al., 1994). The lysine transport system of porcine mammary gland falls into this latter group in substrate specificity. Lysine uptake was inhibited by high concentrations of L-alanine, L-methionine, L-leucine, and cycloleucine (Figure 5). Uptake of the L-lysine was partially inhibited by D-lysine, indicating that the lysine transport is not particularly stereospecific. Inhibition of lysine uptake by neutral amino acids such as alanine, leucine, and cycloleucine suggests an interaction of the neutral amino acids in lysine uptake. However, we have not yet established whether the interaction is competitive or noncompetitive. Nevertheless, such an interaction for leucine in lysine uptake has been demonstrated in rat mammary tissue (Shennan et al., 1994; Calvert and Shennan, 1996). The lack of lysine uptake inhibition by 2-(methyl-amino)-isobutyric acid, a paradigm substrate for system A (Shennan et al., 1997), demonstrates a level of specificity of this lysine transport system. The cationic amino acid uptake system in lactating sow mammary tissue is not via the classic y\(^{\prime}\) system, but may be more closely related to the b\(^{0+}\) or y\(^{\prime}\)L systems. However, the K\(_{m}\) of the lysine transport mechanism in sow mammary tissue is much larger than for either system b\(^{0+}\) or y\(^{\prime}\)L (Devés and Boyd, 1998). The specific identity of the lysine transporter in sow mammary tissue remains to be determined.

The relatively high K\(_{m}\) of the lysine transporter system, compared with physiological blood concentrations of lysine, indicates that the kinetic properties of lysine uptake are not limiting in sow mammary tissue. Further enhancement of lysine utilization by mammary tissue must come from manipulation of blood lysine concentrations or from enhancement of intracellular utilization of lysine for milk protein synthesis. Both approaches are complex.

Nearly 80\% of the lysine in milk proteins is derived from the diet (Koehler et al., 1996), indicating that manipulation of dietary lysine is the most direct route for enhancing blood lysine levels. Lysine is considered the first-limiting amino acid in corn-soybean meal-based diets (NRC, 1998), although several other essential amino acids are also required for milk production (Boyd and Kensinger, 1998). Supplementing diets with synthetic lysine can enhance litter weight gain (Knabe et al., 1996), but that may also result in deficiencies of other amino acids. Amino acid deficiencies caused by selectively increasing dietary lysine can sometimes be corrected by dietary supplementation with other essential amino acids, such as valine (Richert et al., 1996, 1997). These types of observations have contributed to the revised recommendations for dietary lysine and the ratio of valine to lysine in recent estimates of nutrient requirements for lactating sows (NRC, 1998).

Mammary gland takes up considerably more branched-chain amino acids and arginine than are secreted in milk protein (Trottier et al., 1997), suggesting the requirement for these amino acids for mammary function extends beyond milk protein synthesis. Results from the present study indicate that the lysine uptake system is not strictly specific for lysine. Several other amino acids are inhibitory to lysine uptake in the explants from lactating sows. Of particular interest are leucine and arginine, both of which are essential for milk synthesis. Lysine uptake in mammary explants in the present study was inhibited by physiological concentrations of arginine and by supraphysiological concentrations of leucine. It remains to be determined whether lysine uptake in vivo is affected by physiological concentrations of several other amino acids essential for milk synthesis, such as arginine, the branched-chain amino acids, and methionine.

The intracellular fate of lysine in lactating mammary gland is generally considered to be utilization for synthesis of proteins. Lysine output in milk is thought to be closely balanced by uptake (Trottier et al., 1997; Boyd and Kensinger, 1998). However, other amino acids, especially branched-chain amino acids, are oxidized or alternatively metabolized by sow mammary tissue for purposes other than protein synthesis (Richert et al., 1998). Preliminary results from this labora-
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Lactating sow mammary tissue is being oxidized, and that mammary cellular protein synthesis accounts for a significant proportion of total protein synthesis in the tissue (our unpublished observations). Lysine uptake and intracellular utilization need to be more fully defined under conditions that reflect a physiological mix of amino acids.

Implications

Lysine uptake in lactating sow mammary tissue occurs by a sodium-independent transport mechanism with a $K_m$ of approximately 1.4 mM and a $V_{max}$ of 7.9 mmol·kg cell water$^{-1}$·30 min$^{-1}$. Transmembrane transport of lysine by lactating sow mammary tissue should be a simple function of plasma concentrations. Manipulating plasma lysine concentration by nutrition or by altering metabolism of the sow affects lysine uptake by the gland. The lysine transport system in sow mammary gland should not be limiting to milk protein synthesis. Lysine transport is inhibited by high concentrations of a variety of other amino acids, particularly arginine and leucine, both of which are taken up in excess of their secretion in milk during lactation. The potential for competition of lysine uptake by other amino acids needs to be further investigated in cases in which imbalances of plasma amino acids may occur in response to feeding high concentrations of crystalline amino acids.

Literature Cited


