Characterization of Methylaminoisobutyric Acid Transport by System A in Rat Mammary Gland

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During lactation, the mammary gland has a large demand for amino acids for the synthesis of milk proteins and fatty acids. Arteriovenous differences in amino acids across the mammary gland show an elevated uptake of small neutral amino acids that are mainly transported via system A. The purpose of this study was to characterize the transport of methylaminoisobutyric acid (MeAIB), an amino acid analog used to model transport by system A in lactating rat mammary gland explants. MeAIB accumulation in mammary gland cells increased steadily, and after 3 hours of incubation, the intracellular concentration of the analog was 8-fold higher than the concentration in the medium. MeAIB transport into mammary gland explants showed a K_m of 3.3 ± 0.4 mmol/L and a maximal velocity (Vmax) of 555 ± 23 pmol/μL intracellular fluid (ICF) · min, indicating a system with high capacity but low affinity for its substrate. MeAIB transport into mammary tissue depended highly on Na^+, and the uptake was inhibited by addition of natural and analog small neutral amino acids. Cationic, anionic, and large neutral amino acids did not reduce MeAIB transport into mammary gland explants. Preincubation of mammary gland explants in an amino acid–free medium stimulated MeAIB transport, suggesting an adaptive regulation. The addition of an equimolar mixture of alanine, glycine, and serine to the preincubation medium inhibited stimulation of MeAIB transport. Furthermore, stimulation of MeAIB uptake by amino acid starvation was also prevented by the addition of actinomycin D, cycloheximide, tunicamycin, and colchicine. Dibutyryl cyclic adenosine monophosphate (cAMP) increased MeAIB uptake, whereas phorbol 12-myristate 13-acetate (PMA) did not stimulate MeAIB transport. During the first postweaning days, kinetic analyses showed a decrease of 27% in the Vmax. Injection of rat lactating mammary gland mRNA into *Xenopus laevis* oocytes induced expression of the MeAIB transport system; however, the induction was only 83% above background MeAIB uptake. The results of this study provide a partial explanation for the formation of the metabolic pool of small neutral amino acids in the lactating mammary gland.

THE LACTATING MAMMARY gland has a large demand for amino acids to sustain the synthesis of milk protein, oxidation of amino acids (mainly branched-chain amino acids),[1] and synthesis of fatty acids.[2] Arteriovenous amino acid concentration differences across the mammary gland indicate that amino acids are extracted from the blood to the mammary gland. Amino acid uptake into the mammary gland depends on the type of amino acid and the species.[3-5] The capacity of amino acid extraction in the mammary gland depends on specific transport systems localized in mammary cells.[4,6] Identification and characterization of the amino acid transport systems in the mammary gland are required to understand how the supply of amino acids, in part, may regulate the process of milk-protein synthesis and the formation of other milk components.

Arteriovenous differences in amino acids across the mammary gland of the rat indicate uptake of all amino acids, with alanine and glutamine being the most actively transported.[5] These amino acids are preferentially transported by system A in several tissues.[7] This system has been identified in mouse, rat, and bovine mammary tissue.[3,8,9] System A preferentially transports short straight-chain neutral amino acids, as well as the amino acid analog methylaminoisobutyric acid (MeAIB), which is therefore used as the model to identify amino acid transport via system A.[10] Amino acid transport via system A into mammary gland tissue has been characterized by measuring α-amino isobutyric acid (AIB) uptake in the presence of saturating amounts of MeAIB or 2-aminobicyclo (2,2,1) heptane-2-carboxylic acid (BCH), using the latter as a model for the L system. AIB uptake in many tissues, especially the liver, is achieved via the A and alanine, serine, cysteine (ASC) system.[11] AIB in the mammary gland is transported proportionally by the A and L systems.[9] Therefore, it is important to further characterize transport system A of the lactating mammary gland using MeAIB as its specific amino acid probe.

The aim of the present study was to determine the kinetic and ion-dependent parameters of MeAIB transport, as well as the response of system A in the mammary gland to amino acid deprivation and to cyclic adenosine monophosphate (cAMP). The metabolic role of system A is fundamental for the entry of small neutral amino acids that are required not only to sustain adequate rates of protein synthesis but also as a source of precursor molecules for energy and as osmolytes.[7,12] The molecular analysis of system A has been difficult, since several distinct proteins probably contribute to the entire system A transport activity.[13-15] In this study, we also identify system A activity from rat mammary gland by functional expression in *Xenopus* oocytes.

MATERIALS AND METHODS

Animals

Lactating Wistar rats (200 to 250 g) at 12 days postpartum with 8 to 10 suckling pups were used throughout the study. The animals were maintained on a 12-hour light/dark cycle and allowed free access to water and a chow diet. Rats at the end of lactation (21st day postpartum) were separated from their young by removing the pups from the mother's cage, and are referred to as ''postweaning dams.'' Nonpregnant nonlactating rats were used as control rats, and are referred to as

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"virgin rats." This study was approved by the Animal Care Committee of the Instituto Nacional de la Nutrición, México, in accordance with international guidelines for the use of animals in research.

Preparation of Mammary Tissue Explants

Mammary tissue explants were prepared as described previously.[9] Briefly, rats were anesthetized and then killed by decapitation. The mammary gland was immediately removed and placed at 37°C in 10 mL Krebs-Ringer bicarbonate buffer, pH 7.4, which was equilibrated with 95% O_2/5% CO_2 and contained the following (in mmol/L): 118 NaCl, 4.7 KCl, 3.0 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 25 $NaHCO_3$, 0.55 EDTA, and 11 glucose. After removal of the connective tissue, the mammary tissue was diced into 2- to 5-mg explants. The tissue explants were rinsed repeatedly with buffer at 37°C prior to the transport assay. Liver slices were obtained with a tissue slicer and treated similarly to mammary gland explants. Viability of the explants during the incubation periods was verified by electron microscopy.

Transport Assay

Mammary tissue explants were incubated for 15 minutes at 37°C in Krebs-Ringer bicarbonate buffer containing 0.2 mmol/L MeAIB, using L-[1-^{14}C-MeAIB] as a tracer at a final concentration of 0.5 µCi/mL. Incubations were performed with continuous shaking at 60 cpm; the flasks were continuously gassed with 95% O_2/5% CO_2. The tissue was removed and immediately washed 3 times with 3 mL ice-cold incubation medium without MeAIB.[16] The tissue explants were blotted and placed in 0.4 mL tissue solubilizer (Amersham, Arlington Heights, IL) and maintained at room temperature for at least 14 hours. The resulting solution was neutralized with 15 µL glacial acetic acid, and 5 mL scintillation liquid was added. Radioactivity in these samples and aliquots of the incubation media was measured in a Wallac liquid scintillation counter with automatic quench correction (Wallac, Turku, Finland). For competition assays, the natural amino acids alanine, arginine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, histidine, hydroxyproline, isoleucine, methionine, leucine, lysine, phenylalanine, proline, serine, threonine, tryptophan, and valine and the analog amino acids 2-aminobicyclo(2,2,1)heptane-2-carboxylic acid (BCH), homoserine, and MeAIB were usually added individually to the medium at a concentration of 20 mmol/L. The effect of dibutyryl cAMP and phorbol 12-myristate 13-acetate (PMA) was tested by including these compounds in the transport assay.

Extracellular Space Determination

For determination of the extracellular space, [^{3}H]-sucrose was added to the incubation medium and measured as described previously.[16] The total water content of the explants was measured by drying the tissue in a vacuum oven at 100°C until a constant weight was reached. MeAIB transport was calculated[16] and expressed as picomoles per microliter of intracellular water for a given time.

mRNA Preparation

Mammary tissue was homogenized in guanidinium buffer containing 4 mmol/L guanidinium thiocyanate, 25 mmol/L sodium citrate, pH 7.0, 0.1 mol/L 2-mercaptoethanol, and 1% *N*-laurylsarcosine.[17] The homogenate was centrifuged at 12,000 × *g* for 10 minutes at 12°C, and the resulting supernatant was layered onto a cesium chloride solution containing 5.7 mol/L CsCl and 25 mmol/L sodium acetate, pH 5.2. The cesium chloride gradient was formed by centrifugation at 113,000 × *g* for 18 hours at 18°C to yield total RNA. The RNA was washed twice in 75% ethanol and resuspended in RNase-free water. Isolation of poly(A^+) mRNA was obtained by oligo(dT)cellulose chromatography.[17] The poly(A^+) mRNA was precipitated with ethanol, resuspended in RNase-free water, quantified by optical density at 260 nm, and stored at −80°C until use.

Xenopus Oocyte Preparation and Transport Assay

Defolliculated stage VI oocytes were injected with 35 ng rat lactating mammary gland poly(A^+) RNA per oocyte. The oocytes were incubated at 18°C in modified Barth's medium ([MBM] 88 mmol/L NaCl, 1 mmol/L KCl, 2.4 mmol/L $NaHCO_3$, 0.33 mmol/L $Ca(NO_3)_2$, 0.41 mmol/L $CaCl_2$, 0.82 mmol/L $MgSO_4$, 10 µg/mL gentamycin sulfate, 15 mmol/L HEPES, pH 7.6, and 2.5 mmol/L sodium pyruvate) for 5 days. Functional expression of poly(A^+) RNA was assessed by measuring 0.2 mmol/L L-[1-^{14}C]-MeAIB (2 µCi/mL) uptake in groups of 20 to 25 oocytes for 1 hour at 25°C. The Na^+-free medium was prepared by replacing sodium chloride and sodium bicarbonate with the corresponding choline salts. For assays performed in the absence of Na^+, oocytes were first washed in Na^+-free medium for 1 hour before the uptake measurement. After incubation, the oocytes were washed 3 times with ice-cold Na^+-free MBM to remove radiolabeled MeAIB. The oocytes were dissolved in 0.2 mL 10% sodium dodecyl sulfate, transferred to a scintillation vial, and counted in 5 mL scintillation liquid. MeAIB uptake is expressed as picomoles per hour per oocyte.

Statistical Analysis

All assays were performed in triplicate in 2 independent experiments. Results are expressed as the mean ± SE. Data were analyzed by ANOVA followed by Fisher's protected least-significant difference test to determine the significance of differences among groups ($P \leq .05$). Values for the K_m and maximal velocity (Vmax) were obtained by fitting the data to the Michaelis-Menten equation by weighted nonlinear regression analysis.

RESULTS

Time Course of MeAIB Uptake Into the Mammary Gland of Virgin and Lactating Rats and Into the Liver

MeAIB uptake into mammary gland explants of virgin and lactating rats and into the liver was measured for up to 3 hours (Fig 1). MeAIB clearly accumulated in virgin and lactating rat mammary tissue, as its concentration in the intracellular fluid (ICF) was 7.7- and 8.0-fold higher, respectively, than its concentration in the medium after 3 hours of incubation. Uptake of MeAIB into the liver, measured as a reference tissue, was only 2.38-fold higher versus the medium in the same period.

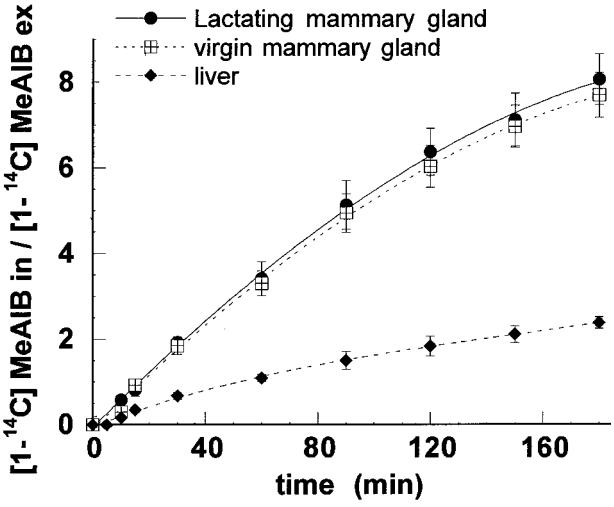

Fig 1. Time course of uptake of 0.2 mmol/L MeAIB in virgin and lactating mammary gland and liver. Results are the mean ± SE.

MeAIB uptake was linear for at least 15 minutes. These results show the high capacity of the rat mammary gland to transport MeAIB with respect to the liver. Rates of MeAIB transport were measured at pH 7.4. MeAIB transport was reduced by acid or alkaline pH. The optimal activity was found at pH 7.4, whereas a pH below 5.5 or above 9.5 inhibited MeAIB uptake into the mammary explants (data not shown).

Kinetics of [1-^{14}C]-MeAIB Uptake

Uptake rates of graded concentrations of MeAIB were measured for 15 minutes in mammary gland tissue and liver. The kinetic characteristics of transport depicted a Michaelis-Menten saturation curve (Fig 2). The K_m for MeAIB was 3.3 ± 0.4 mmol/L with a Vmax of 555 ± 23 pmol MeAIB · µL ICF^{-1} · min^{-1} in the virgin mammary gland, whereas in the lactating mammary gland, the K_m and Vmax were 3.4 ± 0.4 mmol/L and 620 ± 21 pmol MeAIB · µL ICF^{-1} · min^{-1}. The liver showed a Km_m of 3.0 ± 0.3 mmol/L and a Vmax of 210 ± 6 pmol MeAIB · µL ICF^{-1} · min^{-1}.

Effect of Ions on MeAIB Uptake

Maximal MeAIB uptake was only observed in explants incubated with Na^+, independent of the type of anion added (Fig 3A). The substitution of Na^+ with Li^+, Cs^+, choline, or NH_4 decreased the capacity of MeAIB uptake by 32%, 53%, 99%, and 86%, respectively. Substitution of Na^+ and Cl^- with $(NH_4)_2SO_4$, NH_4VO_3, and potassium gluconate decreased MeAIB uptake by 82.4%, 85.8%, and 93.8%, respectively. Kinetic analysis of the Na^+ dependence in mammary gland explants showed a K_m of 2.8 ± 0.2 mmol/L (Fig 3B), which is not different from the K_m measured in liver slices (2.7 ± 0.2 mmol/L; data not shown).

Effect of Amino Acid Competition on MeAIB Uptake

Natural amino acids and amino acid analogs were added to the medium at a concentration of 20 mmol/L, whereas ^{14}C-MeAIB was present at a concentration of 0.2 mmol/L. Transport of MeAIB was not inhibited by arginine, lysine, glutamate, and aspartate, typical anionic and cationic amino acids. The large neutral amino acids phenylalanine, tryptophan, and branched-chain amino acids and the amino acid analog BCH did not depress MeAIB uptake. The addition of threonine, methionine, histidine, hydroxyproline, glutamine, proline, and asparagine reduced MeAIB uptake into mammary tissue by 47% to 71%. Cysteine and the typical small neutral amino acids serine, glycine, alanine, homoserine, and MeAIB were effective competitors of MeAIB uptake (Table 1).

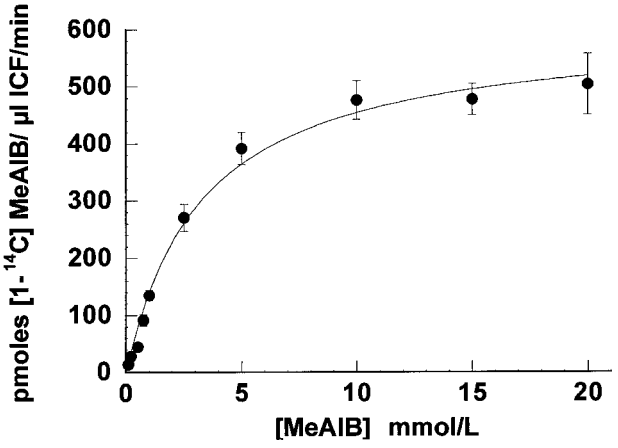

Fig 2. Kinetics of [1-^{14}C]-MeAIB in lactating mammary tissue. Results are the mean ± SE (n = 6).

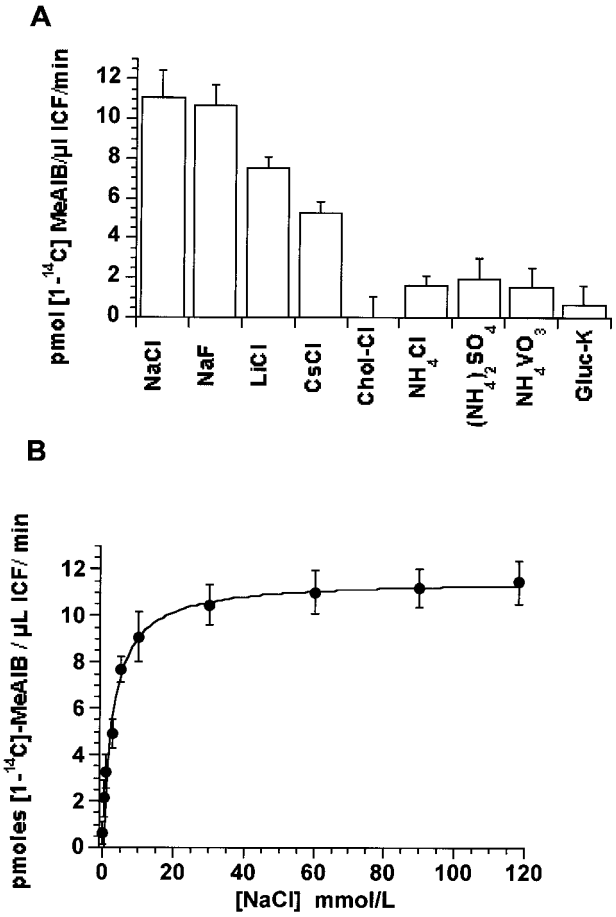

Fig 3. Effect of ions on MeAIB transport in lactating mammary tissue. (A) Effect of ion substitutions. Explants were obtained from mammary gland tissue of dam rats with 12 days of lactation. Explants were incubated with 0.2 mmol/L [1-^{14}C]-MeAIB in Krebs-Ringer bicarbonate-glucose. Isosmolar substitution of NaCl was made with other ion. Results are the mean ± SE (n = 6). Chol-Cl, choline chloride; Gluc-K, potassium gluconate. (B) Kinetic analysis of MeAIB uptake in the presence of graded concentrations of NaCl. Osmolarity in the medium was maintained with choline chloride. Each point represents the mean ± SE (n = 6).

Adaptive Regulation

To study the effect of amino acid starvation on MeAIB uptake, lactating mammary gland explants were first preincubated for different periods in Krebs-Ringer buffer and then transferred to a medium containing 0.2 mmol/L [1-^{14}C]-MeAIB. After 60 minutes of preincubation in an amino acid–free medium, MeAIB uptake increased by 3-fold after 5 hours of preincubation compared with explants without preincubation (Fig 4A). Stimulation of MeAIB uptake by amino acid starvation was partially prevented when mammary gland ex-

Table 1. Effect of Natural and Analog Amino Acids on MeAIB Uptake in Explants of Lactating Mammary Gland

Amino Acid	% of Control
Arginine	109
Isoleucine	106
Glutamate	106
Lysine	103
Phenylalanine	102
BCH	100
Aspartate	97
Valine	95
Tryptophan	94
Leucine	92
Threonine	53
Methionine	42
Histidine	36
Hydroxyproline	35
Glutamine	34
Proline	34
Asparagine	29
Cysteine	20
Serine	20
Glycine	15
MeAIB	5
Alanine	2
Homoserine	0

plants were preincubated in a medium containing a mixture of phenylalanine, valine, and lysine. The addition of a mixture of small neutral amino acids (alanine, glycine, and serine) to the preincubation medium inhibited the stimulation of MeAIB uptake. Preincubation of the explants for 5 hours in an amino acid–free medium containing the inhibitors of RNA and protein synthesis, actinomycin D and cycloheximide, blocked the stimulation of MeAIB uptake. The glycosylation inhibitor tunicamycin partially inhibited the stimulation of MeAIB uptake. The antimitotic agent colchicine, which depolymerizes microtubules, not only blocked the stimulation of MeAIB uptake but also decreased the transport activity of MeAIB compared with explants without preincubation.

Effect of Dibutyryl cAMP and PMA on MeAIB Uptake

The addition of 1 mmol/L dibutyryl cAMP significantly increased the transport of MeAIB by 85% with respect to the control group. However, PMA (shown as TPA in Fig 5) at the same concentration did not change MeAIB uptake into mammary gland explants (Fig 5). Lower concentrations of PMA did not change the rate of MeAIB uptake (data not shown).

Effect of Weaning on K_m and Vmax

Table 2 shows the kinetic constants of MeAIB transport in mammary gland explants at the peak of lactation and 1, 3, 5, 8, and 12 days postweaning. The K_m value remained unchanged during the periods assessed. However, the Vmax showed a significant reduction of about 50% 1, 3, and 5 days postweaning compared with the peak of lactation. On day 12 postweaning, the Vmax was only 19% lower than the value observed during lactation, and it almost reached the estimated Vmax measured in mammary gland explants of virgin rats.

Expression of MeAIB Transporter in Xenopus Laevis Oocytes

mRNA isolated from the mammary gland during lactation and from the liver was injected into *Xenopus laevis* oocytes. Maximal expression was obtained 5 days after injection of oocytes with mRNA. Injection of mRNA into the oocytes increased the uptake of MeAIB 7.6-fold in the presence of sodium as compared with oocytes injected with water. Oocytes injected with mRNA in the presence of sodium had a 3.8-fold higher MeAIB uptake rate than those incubated in a sodium-free medium (Fig 6).

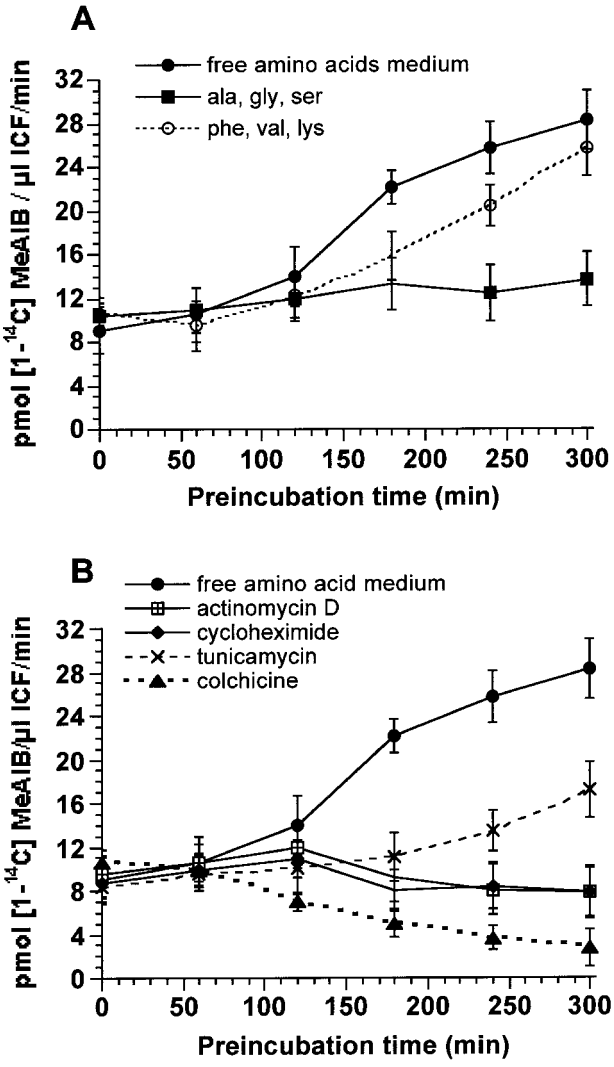

Fig 4. Effect of preincubation time in amino acid–free medium on uptake of MeAIB by lactating mammary gland. (A) Addition of a mixture of small neutral amino acids (alanine, glycine, and serine, 1 mmol/L each) or a mixture of phenylalanine, valine, and lysine, 1 mmol/L each. Uptake rates of 0.2 mmol/L [1-^{14}C]-MeAIB were measured after 15 minutes of incubation. (B) Addition of 50 µmol/L actinomycin D, 0.1 mmol/L cycloheximide, 0.2 mg/mL tunicamycin, and 5 µmol/L colchicine on MeAIB uptake in explants of lactating mammary tissue preincubated for 5 hours in amino acid–free medium. Each point represents the mean ± SE (n = 6).

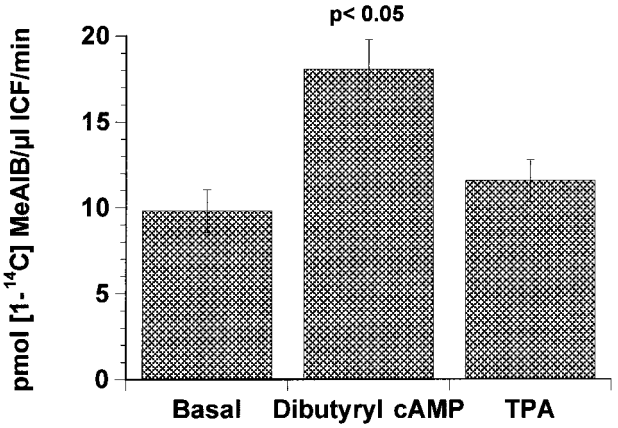

Fig 5. Effect of dibutyryl cAMP and PMA (TPA) on MeAIB uptake in explants of lactating mammary gland. Explants were preincubated for 1 hour in Krebs-Ringer bicarbonate buffer containing 1 mmol/L dibutyryl cAMP or 1 mmol/L PMA. Transport of 0.2 mmol/L [1-^{14}C]-MeAIB was measured after 15 minutes. Each bar represents the mean ± SE (n = 6).

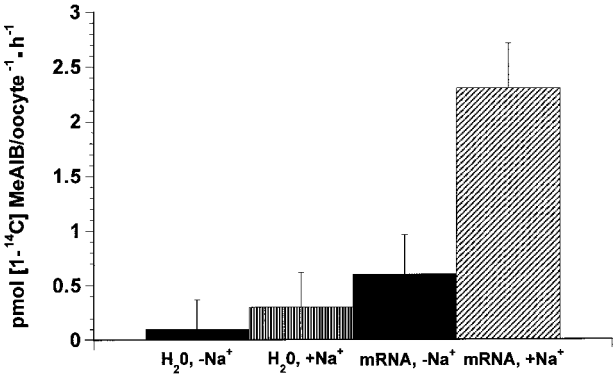

Fig 6. Expression of MeAIB transporter in *Xenopus* oocytes injected with mRNA isolated from lactating mammary gland. MeAIB transport was assessed by measuring the uptake of 0.2 mmol/L L-[1-^{14}C]-MeAIB for 1 hour at 25°C. Sodium salts were substituted with the respective choline salts. Each bar represents the mean ± SE of 4 experiments.

DISCUSSION

The transport of amino acids into the mammary gland during lactation is an active process that maintains the accelerated rate of protein synthesis in this tissue[18,19] and provides specific amino acids, such as branched-chain amino acids, that are catabolized actively in the mammary gland.[1,20] Competition for the entry of amino acids into several tissues limits the metabolic utilization of amino acids, since some of them share the same transport system.[21] This implies that amino acid transport plays an important role in amino acid metabolism in each tissue.

The kinetic characteristics of some amino acid transport systems, including systems X_{AG}, β, ASC, L, and y^+, have been studied in the mammary gland.[4,6] However, there are few studies at the molecular level on the expression of transporter proteins involved in these transport systems. Recently, it has been observed that the expression of the transporters for anionic amino acids, GLAST and GLT-1,[22] cationic amino acids, CAT-1, and β-amino acids (Torres N, Mata DA, De Santiago AR, unpublished results, June 1998) in the mammary gland of the rat is regulated during pregnancy, lactation, and weaning. However, the molecular mechanisms for the expression and regulation of system A are not well understood. Injection of rat mammary gland mRNA into *Xenopus laevis* oocytes modestly increased MeAIB transport uptake by 83% (Fig 6). Studies on the functional expression of transport system A with oocytes injected with mRNA from other tissues also show low-level expression compared with the response observed for other transport systems.[23] In part, this result has made it difficult to clone the cDNA of the transporter protein for this system, since system A is probably formed by several proteins or requires some proteins that are regulators of system A activity.[7] Disarrangement of cytoskeletal proteins with colchicine not only prevented adaptive regulation but also depressed the basal activity of system A in mammary gland explants (Fig 4B). Hepatic system A is associated with integral proteins of the cell membrane, α-fodrin and ankyrin. Therefore, despite our efforts to isolate the protein or proteins involved in the formation of system A, more research is required to identify all of the components.

Table 2. Kinetic Constants for MeAIB Transport in the Rat Mammary Gland During Lactation and Weaning

Postweaning Day	K_m (mmoL/L)	Vmax (pmol MeAIB/µL ICF · min)
0 (lactation)	3.4 ± 0.4	620 ± 21
1	3.1 ± 0.3	311 ± 9.9
3	3.3 ± 0.4	321 ± 12
5	3.3 ± 0.2	342 ± 7
8	3.3 ± 0.3	412 ± 12
12	3.2 ± 0.4	503 ± 20
Virgin rat	3.3 ± 0.4	555 ± 23

MeAIB transport occurred actively in the mammary gland in nonpregnant and lactating rats. Uptake of MeAIB via system A showed saturable kinetics and also the capacity to concentrate this model amino acid analog in the mammary gland (Figs 1 and 2). Kinetic parameters of MeAIB uptake have been estimated in different tissues and organs. Although the overall affinity for MeAIB in the mammary gland was relatively low (3.4 ± 0.4 mmol/L) as compared with the heart (1.1 ± 0.03 mmol/L),[24] K_m values in the mammary gland were similar to those observed in the liver (3.0 ± 0.3 mmol/L) and muscle (3.2 ± 0.2 mmol/L).[16] Vmax values for MeAIB in lactating mammary tissue were higher (620 ± 21 pmol MeAIB · µL ICF^{-1} · min^{-1}) than the values observed in liver, heart, and muscle.[16,24] These results suggest that tissues with an elevated requirement for amino acids show a high capacity for amino acid transport.

The transport of MeAIB was inhibited by the addition of 20 mmol/L small neutral amino acids, but not by anionic, cationic, or large neutral amino acids. The high concentration used for the amino acid competitors allows one to distinguish which amino acids inhibit MeAIB transport by saturating transport

system A. However, under physiological conditions, the total plasma concentration of small neutral amino acids is in the range of the K_m determined for system A in the mammary gland, which presumably can predict a certain degree of competition for their entry into mammary gland tissue. Preincubation of explants of mammary tissue in amino acid–free medium induced transport system A via an adaptive regulation process similar to that observed in other tissues or cells, including the liver and skeletal muscle.[11,16,25-28] The addition of a mixture of small neutral amino acids transported by system A to the preincubation medium inhibited the stimulation of MeAIB uptake. Furthermore, stimulation of MeAIB transport in rat mammary gland explants incubated in an amino acid–free medium was blocked by inhibitors of RNA and protein synthesis, indicating that the stimulation of transport activity is dependent on the synthesis of the transporter protein or of a regulatory protein for system A activity.

The regulation of amino acid uptake activity into the mammary gland is probably controlled by hormones; prolactin, in particular, stimulates the transport of most amino acids into the lactating mammary gland.[29] In this study, we did not observe differences in the K_m and Vmax during lactation. However, there was a significant reduction in the Vmax during the first 5 days of the postweaning period (Table 2), probably associated with a decline of prolactin levels[30] and the involution process occurring in the mammary gland of postweaning dams. On the other hand, unpublished results for our laboratory have demonstrated that during the weaning period, there is a reduction in the concentration of mRNA for the transporters CAT-1 and BETA involved in the transport of cationic and β-amino acids.

Previous studies in hepatocytes showed that system A activity is induced by glucagon or by the cAMP analog dibutyryl cAMP. Mammary gland explants also showed an increase in MeAIB transport when incubated in a medium containing dibutyryl cAMP; however, PMA did not affect system A activity, indicating that the activation of proteins that form system A is mediated, in part, via protein kinase A.

The characterization of transport system A allows a partial explanation for the preservation of the metabolic pool of small neutral amino acids in the mammary gland during lactation. The cloning of a specific cDNA for the system A transporter protein will provide a tool to study changes in the gene expression of system A.

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