Mechanism and Regulation of Amino Acid Transport in Mammary Gland - Review -

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ABSTRACT : Several amino acid transport systems in mammary gland have been characterized during the last few years. These systems may be divided into two broad categories based on whether they are sodium-dependent or Na^+-independent, and each of these categories is subdivided into 3 groups depending on whether the systems prefer zwitterionic, cationic or anionic substrates. The zwitterion preferring transport processes in mammary gland are Na^+-dependent system A and Na^+-independent systems L and T. System y^+ is a Na^+-independent transporter of cationic amino acids and X_{AG}^- is a Na^+-dependent system for anionic amino acids. A (Na^++Cl^-)-dependent system, selective for β-amino acids has been reported in rat mammary tissue. In addition, there is yet another class of transporters that have still broader specificity. The Na^+-dependent systems BCl^--dependent and BCl^--independent and Na^+-independent system y^+L have been reported to mediate the transport of zwitterionic as well as cationic amino acids. Each system has been characterized with respect to its substrate specificity, affinity, kinetics and ion-dependence. Transport of amino acids by mammary tissue is regulated by i) the intracellular substrate concentration, ii) lactogenic hormones and iii) milk stasis. Four of the above transport systems (i.e. A, L, y^+ and BCl^--independent) are up-regulated by lactogenic hormones (insulin, cortisol and prolactin) in mammary gland. (*Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 5 : 710-719*)

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INTRODUCTION

The lactating mammary gland has a large requirement for amino acids to meet the need of milk protein synthesis. Amino acids are extracted by transport systems situated on the blood facing aspect of the mammary epithelial cells, as reflected by the large arterio-venous amino acid concentration differences across the gland (Metcalf et al., 1991; Guinard and Rulquin, 1995). Identification and characterization of these transport systems and elucidation of the mechanism of amino acid entry into the mammary gland will help understand the process of milk protein synthesis and secretion. The transport of certain amino acids by mammary secretory cells could be rate limiting for milk protein synthesis (Mepham, 1982). Although an increase in milk protein concentration has been established on certain diets, relatively little information has been produced on the mechanism by which increases in some of these trials have been achieved. Consequently such manipulations have poor predictability of response for increasing milk protein content. In this connection many studies have attempted to identify rate limiting amino acids by way of manipulating plasma amino acid concentrations (Shennan et al., 1997). This approach has often met with little success and probably reflects upon the lack of knowledge about substrate specificity and kinetics of mammary amino acid transporters.

Most mammalian cells express a common 'core' set of amino acid transport activities, but all also exhibit wide variations in the type and activity of these transport systems (Kilberg et al., 1993). As a result, each cell type is unique with regards to the processes available for amino acid accumulation, and has adapted to its physiological role and metabolic needs. A number of reviews have covered amino acid transport systems that exist in many mammalian cell types and have described individual transport activities and the associated regulation (VanWinkle, 1988; Christensen, 1990; Kilberg et al., 1993; Tunnicliff., 1994; MicGiven and Pastor-Anglada, 1994; Bertran et al., 1994; Malandro and Kilberg, 1996; Deves and Boyd, 1998). The information on the mechanism of amino acid transport in mammary gland has appeared recently. The objective of this paper is to review current knowledge about amino acid transport mechanism in lactating mammary gland.

APPROACH FOR DISCRIMINATION AND CHARACTERIZATION OF AMINO ACID TRANSPORT SYSTEMS

The amino acid transporters have broad specificity, and the transport of each amino acid is mediated via more than one transport systems. Except for A and L systems, there is no specific model substrate for amino acid transporters. The heterogeneity in transport modes and the lack of specific model substrate has made identification and discrimination of amino acid

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transporters a difficult task. Often a multifarious approach is adopted to identify the transport system on the basis of following criteria:

* Competition between amino acids for the transporter
* Affinity of the transporter for amino acid analogues, 2-aminobicyclo(2,2,1) heptane-2-carboxylic acid(BCH) and 2-(methylsmino) isobutyric acid (MeAIB)
* Dependence of the transport systems on Na^+ or Na^+ plus Cl^- or none
* Sensitivity of the amino acid transport to H^+ concentration of the extracellular medium
* Sensitivity of the amino acid transporter to *N*-ethylmaleimide
* Trans-acceleration or down-regulation of amino acid flux by intracellular accumulation of substrate amino acids
* Kinetic parameters (K_m and V_{max})
* Regulation of transport systems by lactogenic hormones.

Several amino acid transport systems in mammary gland have been characterized in the recent past. These systems may be divided into two broad categories based on whether they are Na^+-dependent or Na^+-independent, and each of these categories is subdivided into 3 groups depending on whether the systems prefer cationic, zwitterionic or anionic substrates. There is yet another class of transporters that have still broader specificity and interact with zwitterionic as well as cationic amino acids.

TRANSPORT SYSTEMS SPECIFIC FOR ZWITTERIONIC AMINO ACIDS

The zwitterion specific transport processes identified in mammary gland are Na^+-dependent system A and Na^+-independent systems L and T.

System A

System A, which is characterized by its tolerance of N-methylated substrate (e.g., N-methylamino-isobutyric acid) has been described in mouse, rat and bovine mammary tissue (Neville, et al., 1980; Baumrucker, 1985; Verma and Kansal, 1993, 1995; Shennan and McNeillie, 1994; Sharma and Kansal, 1999). System A is energized by the Na^+-gradient across the cell membrane, maintained by the action of the Na^+-K^+ pump. The mammary A system has high affinity for short straight chain neutral amino acids (L-alanine, glycine, alpha-aminoisobutyric acid, L-serine etc.) and low affinity for long chain aliphatic amino acids (L-methionine, L-leucine etc.). This system does not interact with aromatic amino acids (Neville et al., 1980; Verma and Kansal, 1993; Rekha and Kansal, 1996a; Sharma and Kansal, 1999). The activity of system A is responsible for the intracellular accumulation of short straight chain amino acids in the mammary tissue. The V_{max} for L-alanine is more than 3 times of that for L-methionine transport in mouse mammary tissue (Verma and Kansal, 1993; Sharma and Kansal, 1999). System A is highly sensitive to low pH. The uptake of L-alanine (Sharma and Kansal, 1999) and glycine (Rehan et al., 2000) via system A was observed to decrease sharply at pH 6.0. System A is down regulated by intracellular accumulation of its substrate amino acids. Prior loading of mammary cells with neutral amino acids was reported to diminish the A system mediated uptakes of L-alanine and glycine (Sharma and Kansal, 1999; Rehan et al., 2000). System A is also sensitive to *N*-ethylmaleimide, suggesting the role of sulfhydryl groups in the transport of amino acids via this system. Treatment of mammary cells with *N*-ethylmaleimide has been reported to abolish completely the uptake of glycine via system A (Rehan et al., 2000). Sharma and Kansal (1999) have shown the dependency of system A on Na^+ plus Cl^- for the transport of L-alanine into mouse mammary tissue. For the uptake of glycine, however, system A in mouse mammary tissue has been resolved into two components, one dependent on Na^+ plus Cl^- and the other on Na^+ alone (Rehan et al., 2000).

System L

The presence of system L in lactating mammary tissue has been shown in the mouse, rat, guinea pig and cow (Neville et al., 1980; Baumrucker, 1985; Mepham et al., 1985; Shennan et al., 1994a). The uptake of amino acids by mammary tissue explants isolated from rat and mouse is inhibited by 2-(aminobicyclo)heptane-2-carboxylic acid (BCH), a paradigm substrate of the system L (Neville et al., 1980; Verma and Kansal, 1993; Shennan et al., 1994a; Rekha and Kansal, 1996a; Sharma and Kansal, 1999). System L has specificity for most naturally occurring neutral amino acids, with higher affinity for long and branched-chain aliphatic and aromatic amino acids (e.g. L-methionine, L-leucine, L-tyrosine, L-tryptophan etc.). In studies involving isolated mouse mammary tissue explants, the K_m values for L-alanine, L-methionine and L-tyrosine were observed 1.98, 0.46 and 0.23 mM respectively, and V_{max} 11.2, 30.0 and 31.0 nmol/g cells/min, respectively (Verma and Kansal, 1995; Rekha and Kansal, 1996a; Sharma and Kansal, 1999). System L appears to act as an exchange mechanism for amino acid uptake across the membrane. Prior loading of mouse mammary cells with L-methionine produced 2.5 fold stimulation in its uptake via the L system (Rekha and Kansal, 1996a). Also, BCH-inhibitable uptake of L-[^{14}C]tyrosine was *trans*-accelerated by intracellular L-tyrosine.

Baumrucker (1985) has observed similar results with the uptake of L-leucine, a specific system L substrate, by bovine mammary tissue explants. The transport of amino acids via the L system is inhibited by thiol reagent, *N*-ethylmaleimide (Rekha and Kansal, 1996a).

System T

Rekha and Kansal (1996a) have established the presence of system T in mouse mammary gland. The T system is a low affinity transporter, which interacts with only aromatic amino acids. The K_m value of system T mediated uptake of L-tyrosine in mouse mammary tissue explants is 15.7 mM (Rekha and Kansal, 1996a). At physiological concentrations, the aromatic amino acids are largely transported via the L and broad specificity systems. Only at higher substrate concentrations, the T system accounts for the significant uptake of aromatic amino acids by mammary tissue. Like other zwitterionic amino acid transporters (A and L), the T system is also inhibited by thiol reagent *N*-ethylmaleimide. Rekha and Kansal (1996a) observed no change in the influx of amino acids via system T into mouse mammary tissue explants loaded with L-tyrosine, which suggests that the T system is neither down-regulated nor *trans*-accelerated by intracellular accumulation of its substrate. System T was earlier described only in human erythrocytes (Vadgama and Christensen, 1985) and rat hepatocytes (Salter et al., 1986).

System β

Taurine (2-amino ethanesulfonic acid) is a β-amino acid, which is involved in a large variety of biological functions. The mammary glands of a number of species are capable of concentrating taurine in milk (Rassin et al., 1978). The uptake of taurine by lactating rat mammary tissue explants is dependent on the presence of Na^+ and Cl^- (Shennan and McNeillie, 1994b). This transport mechanism is a high affinity system (K_m 43 μM), which only accepts β-amino acids (i.e., taurine, β-alanine, hypotaurine) as substrates. The mammary β-amino acid transport system is similar to that described in placenta, intestine and kidney (Huxtable, 1992). A high affinity (K_m 70 μM) Na^+-dependent transport system selective for β-amino acids also has been described in gerbil mammary gland (Shennan, 1995). However, an unusual property of taurine uptake by gerbil mammary tissue is that it is not dependent on Cl^-.

TRANSPORT SYSTEMS FOR CATIONIC AMINO ACIDS

Baumrucker (1984) showed that the transport of the cationic amino acids L-arginine and L-lysine into bovine mammary tissue occurred by a single specific Na^+-independent route with characteristics similar to the y^+ system reported in other tissues (White and Christensen, 1982; Kilberg et al., 1993). A Na^+-independent system that transports cationic amino acids and also interacts with the neutral amino acids L-leucine and L-glutamine was reported in rat mammary tissue (Shennan et al., 1994b). A wide range of neutral amino acids, in addition to cationic amino acids, are capable of inhibiting L-lysine uptake by, and stimulating L-lysine efflux from rat mammary tissue explants. Later, Calvert and Shennan (1996) appeared to rule out the y^+ system as the mechanism responsible for L-lysine transport in rat mammary tissue.

Sharma and Kansal (2000) have shown the presence of two Na^+-independent systems in mouse mammary tissue for the transport of cationic amino acids, which are distinguishable on the basis of their sensitivity to L-leucine. The leucine-sensitive uptake of L-arginine (K_m 0.4 mM) is through a broad specificity system similar to the y^+L system reported in human erythrocytes (Deves et al., 1992) and placenta (Eleno et al., 1994; Furesz et al., 1995). The broad specificity system interacts with both cationic and neutral amino acids and is inhibited by preloading mammary tissue with neutral amino acids. The leucine-insensitive uptake of L-arginine in mouse mammary tissue (Sharma and Kansal, 2000) is identified as the y^+ system (K_m 0.76 mM). Intracellular accumulation in mammary tissue of cationic but not neutral amino acids increases the uptake of cationic amino acids via the y^+ system. The y^+ system activity is suppressed by decrease in pH of the extracellular medium.

Assuming that both the y^+ and y^+L systems are in the basolateral membrane of mammary epithelial cells, the y^+ system being specific for cationic amino acids appears to be the main transporter of L-arginine. The influx of cationic amino acids via the y^+L system would be restricted owing to competition with neutral amino acids. Furthermore, the broad specificity system y^+L is down regulated by the accumulation of neutral amino acids. Its contribution to cationic amino acids uptake would decrease with increased intracellular neutral amino acid concentrations, where as the influx via system y^+ is accelerated by the intracellular accumulation of cationic amino acids.

The values of the kinetic constants (K_m and V_{max}) of cationic amino acid transport systems are greater in the lactating mammary gland (Sharma and Kansal, 2000) than in many other tissues (Deves and Boyd, 1998). This natural adaptation ensures a more rapid uptake of amino acids by lactating mammary gland after food intake, when plasma amino acid concentrations are raised, and a slower uptake when they are lower between meals. The greater K_m values of amino acid transporters in lactating mammary gland

than in other vital organs also ensure that amino acids are extracted in preference by the vital body organs at low concentrations of amino acids in blood. Consistent with the lower demand for amino acids by non-lactating mammary gland, the V_{max} of transport systems is smaller in pregnant than in lactating mammary tissue (Verma and Kansal, 1993). Further, the transport systems y^+, A, L and BCl^--independent remain suppressed in non-lactating mammary tissue and are upregulated by lactogenic hormones at the onset of lactation (Sharma and Kansal, 1999, 2000).

TRANSPORT SYSTEMS FOR ANIONIC AMINO ACIDS

Three transport systems capable of transporting anionic amino acids have been characterized in mammalian cells. These are i) a high affinity (K_m 1-290 μM) Na^+-dependent system X_{AG}^- (Dall'Asta et al., 1983), ii) a low affinity (K_m 2-4 mM) Na^+-dependent system identified in the fibroblasts (Dall'Asta et al., 1983), hepatocytes (Kilberg et al., 1993) and intestine (Wingrove and Kimmich, 1988) and iii) a Na^+-independent system xc^- capable of transporting L-glutamate and cystine (Bertran et al., 1994).

A system similar to X_{AG}^- has been reported to mediate the transport of anionic amino acids in rat (Millar et al., 1996a) and mouse (Kansal et al., 2000) mammary gland. The anionic system in mammary tissue is Na^+-dependent and stereo-specific with respect to glutamate but not aspartate. It interacts with both D- and L- isomers of aspartate, but only with L^- isomer of glutamate. Replacement of Cl^- by gluconate from the extracellular medium does not affect the uptake of L-glutamate by mouse mammary tissue explants (Kansal et al., 2000). Although neutral amino acids weakly inhibit the uptake of L-glutamate, there is no evidence for the heterogeneity of anionic amino acid transporter in mouse mammary gland (Kansal et al., 2000). The X_{AG}^- transporter of mouse mammary gland is highly sensitive to sulfhydryl group blocking agent *N*-ethylmaleimide, and partially inhibited at low pH (6.0) of the extracellular medium (Kansal et al., 2000).

L-glutamate transport by mammary gland is a high affinity system. The K_m values of L-glutamate uptake found with rat mammary explants and the perfused rat mammary gland are respectively 118 and 18 μM (Millar et al., 1996a), the difference between the two values probably reflects the presence of larger unstirred fluid layer associated with the explants (Barry and Diamond, 1984).

Some of the high affinity glutamate transporters from non-mammary tissues have been cloned and their primary amino acid sequences have been determined (Kanai and Hediger, 1992; Pines et al., 1992; Strock et al., 1992). Glutamate and aspartate uptake by rat mammary tissue is inhibited by dihydrokainate but not aminoadipate (both are L-glutamate analogues), this pharmacological profile is not exhibited by any of the cloned glutamate transporters, thus mammary gland expresses a novel from of glutamate transporter (Millar et al., 1996b).

To look for the possibility of a low affinity Na^+-dependent system for L-glutamate transport, Kansal et al. (2000) determined the effect of L-serine on the uptake of L-glutamate by mouse mammary tissue explants in the presence of system X_{AG}^- saturating concentration of D-aspartate under slightly acidic conditions (pH 6). Low pH was selected keeping in view of the observation that ASC like activity may serve upon protonation as a low affinity pathway for anionic amino acids (Maenz et al., 1992). The choice of inhibitory amino acid was dictated by the fact that the X_{AG}^- system displays no stereo-specificity for aspartate, whereas, ASC-like system excludes D-aspartate (Christensen, 1984). L-serine was chosen for being a preferred substrate of the ASC system. Kansal et al. (2000) observed no significant inhibition of L-glutamate uptake by L-serine in the presence of 5 mM D-aspartate at pH 6, hence it rules out the possibility of low-affinity Na^+-dependent glutamate transporter in mouse mammary gland (table 1).

At physiological concentration, over 90% of L-glutamate uptake by mouse mammary tissue is via Na^+-dependent system X_{AG}^-. The Na^+-independent uptake of L-glutamate is linear with respect to its external concentration, and is not inhibited by any

Table 1. Effect of D-aspartate and L-serine on the uptake of L-glutamate at pH 6.0* [values are nmol/10 min/g cells, mean ± SEM, n=4]

Competing amino acids		
D-aspartate (mM)	L-serine (mM)	L-glutamate uptake
0.0	0	49.3 ±3.3
0.05	0	42.0 ±2.2
0.2	0	26.0 ±12.7
0.5	0	17.3 ±1.9
2.0	0	11.4 ±0.8
5.0	0	11.0 ±0.2
5.0	1	7.0 ±0.8
5.0	2	6.9 ±0.2
5.0	5	8.9 ±0.7
5.0	10	6.9 ±0.5
5.0	20	8.1 ±0.3

* L-glutamate concentration of the external medium was 0.05 mM in buffer containing Na^+ and Cl^-. [Adapted from Kansal et al. (2000)].

amino acid, which rules out the possibility of any Na^+-independent system as the mechanism for L-glutamate transport in mouse mammary tissue (Kansal et al., 2000).

BROAD SPECIFICITY SYSTEMS FOR THE TRANSPORT OF AMINO ACIDS

A Na^+-dependent, MeAIB insensitive transport of neutral amino acids has been described in bovine (Baumrucker, 1984) and murine (Verma and Kansal, 1993) mammary gland. Sharma and Kansal (1999) have shown that Na^+-dependent, MeAIB-insensitive transport of amino acids in mouse mammary tissue is comprised of two broad specificity transporters, which they named BCl^--dependent and BCl^--independent. In addition a Na^+-independent broad specificity system y^+L has been described in mouse mammary gland (Sharma and Kansal, 2000). The transport of amino acids in lactating mouse mammary tissue mediated by the BCl^--dependent system depends on Na^+ and Cl^-. The BCl^--dependent system interacts with a variety of amino acids including L-serine, L-alanine, D-alanine, BCH and L-arginine, hence it exhibits broad specificity. The influx of L-alanine via BCl^--dependent system is *trans*-accelerated in mammary tissue preloaded with neutral but not cationic amino acids (Sharma and Kansal, 1999; table 2). BCl^--independent system requires Na^+ but not Cl^-, and is a broad specificity system that interacts strongly with L-alanine, L-serine and L-arginine and moderately with BCH and D-alanine. It differs from BCl^--dependent system in its affinity for different substrates and lack of requirement for Cl^-. The BCl^--independent system is strongly down-regulated by intracellular accumulation of neutral as well as cationic amino acids. Prior loading of mammary cells with either L-alanine or L-arginine completely abolishes the uptake of L-alanine via the BCl^--independent system (Sharma and Kansal, 1999; table 2).

AMINO ACID TRANSPORT SYSTEMS ABSENT IN MAMMARY TISSUE

The ASC system is a major component of Na^+-dependent transport of amino acids in rabbit intestine, rabbit reticulocytes, pigeon erythrocytes, rat hepatocytes, cultured human fibroblasts, HTC hepatoma cell line and Chinese hamster ovary cells etc. (Lazarus and Panasci, 1986). The ASC system, unlike system A, cannot transport N-methylated amino acids. The ASC system prefers linear dipolar amino acids, such as L-alanine, L-serine and L-cysteine, and has relatively higher affinity for amino acids containing distal hydroxyl group (Kilberg et al., 1981). A Na^+-dependent, MeAIB-insensitive transport of neutral amino acids has been described in bovine (Baumrucker, 1984) and murine (Verma and Kansal, 1993) mammary gland, and has been referred to as the ASC system. Sharma and Kansal (1999) have shown that the Na^+-dependent, MeAIB-insensitive transport of neutral amino acids in mouse mammary tissue is mediated by two broad specificity systems, and ruled out the ASC system in this tissue. Neville et al. (1980) found no evidence for the Na^+-dependent, MeAIB-insensitive uptake of α-aminoisobutyric acid by mouse mammary tissue explants. This would suggest that the broad specificity systems do not accept α-aminoisobutyric acid as substrate.

The Na^+-independent asc system, analogous in substrate specificity to Na^+-dependent system ASC with a high affinity for L-alanine, has been characterized in horse (Fincham et al., 1988) and pigeon (Vadgama and Christensen, 1985) erythrocytes. Sharma and Kansal (1999) observed that the inhibitions of L-[^{14}C]alanine influx into mouse mammary tissue explants, caused by BCH and unlabelled L-alanine were of the same order. Furthermore, the Na^+-dependent, BCH-insensitive uptake was linear with increasing external substrate concentration. These observations rule out system ASC as the mechanism responsible for neutral amino acid transport in mouse mammary gland.

System N identified in rat liver (Kilberg et al., 1980; Low et al., 1991) has a narrow substrate specificity, being restricted to amino acids with nitrogen containing side chain such as L-glutamine, L-aspargine and L-histidine. Rekha (1998) has shown that the uptake of L-glutamine by mouse mammary tissue explants is mediated by four systems: (i) system A, (ii) (Na^++Cl^-)- dependent, MeAIB-insensitive broad specificity system, (iii) Na^+-dependent, Cl^--independent, MeAIB-insensitive broad specificity system and (iv) system L. System N in liver is dependent on Na^+ and does not interact with MeAIB. The Na^+-dependent, MeAIB-insensitive uptake of L-glutamine in mouse mammary tissue is not affected by L-histidine (Rekha, 1998). L-histidine is a preferred substrate of system N in rat liver (Ahmed et al., 1990). Absence of system N in mouse mammary tissue is further confirmed by the observa- tion that the uptake of L-glutamine via Na^+-dependent, MeAIB-insensitive systems is increased at pH 6.0 (Rekha, 1998), whereas, the N system activity in rat hepatocytes diminishes at this pH (Kilberg et al., 1980, 1981). Furthermore, the uptake of L-glutamine via the Na^+-dependent, MeAIB-insensitive systems is inhibited following starvation of mammary tissue for amino acid (Rekha, 1998), whereas, the N system in rat hepatocytes is upregulated by starvation for its substrate amino acids (Kilberg, 1982; Handlogten et al., 1982).

The glycine transporter is a Na^+-dependent system

Table 2. Effect of loading lactating mouse mammary tissue with amino acids on systems BCl^--dependent and BCl^--independent of L-alanine uptake (Mean values ± SEM, n=4, nmol min^{-1} g^{-1} cell)

Amino acid loaded	BCl^--dependent†	BCl^--independent‡
None (control)	3.51 ± 0.29	1.84 ± 0.13
L-alanine	8.98 ± 0.30**	0
L-arginine	3.56 ± 0.16	0

Prior to amino acid uptake measurement, mammary tissue was incubated in Na^+ plus Cl^--buffer containing indicated amino acid (10 mM) for 30 min at 37°C in shaking water bath (90 cycles/min). The control tissue was incubated in the buffer without amino acid. At the end of loading period, tissue slices were washed with the buffer (ice-cold) used for uptake studies. The uptakes of L-alanine (from 0.1 mM external L-alanine) by mammary tissue were determined in media containing (1) Na^+, Cl^- and 10mM-MeAIB; 2) Na^+ and 10mM-MeAIB; and (3) Cl- but no Na^+. † Difference of L-alanine uptakes in media 1 and 2; ‡ Difference of L-alanine uptakes in media 2 and 3. **($p>0.01$) significantly different from their control values. [Adapted from Sharma and Kansal, (1999)].

selective for glycine, its methyl derivative sarcosine and proline. It has been reported in pigeon (Imler and Vidaver, 1972) and human (Ellory et al., 1981) erythrocytes, rat liver (Christensen and Handlogten, 1981; Kilberg, 1982), rat spinal cord (Lopez-Corcuera and Aragon, 1989) and mouse eggs and pre-implantation embryo (Hobbs and Kaye, 1985; VanWinkle, 1988). The glycine system appears to be absent in mouse mammary gland. Rehan et al. (2000) have shown that glycine transport in lactating mouse mammary gland in mediated by three Na^+-dependent systems: i) the A; ii) (Na^++Cl^-)-dependent, MeAIB-insensitive; and iii) Na^+-dependent Cl^--independent MeAIB-insensitive. While the A system transports neutral amino acids, the latter two are broad specificity systems.

Gamma glutamyl cycle

Gamma-glutamyl transpeptidase (GGT), a membrane bound glycoprotein enzyme, in conjunction with the gamma-glutamyl cycle was proposed to constitute a system for the transport of amino acids in kidney and other cells (Meister, 1988). Its involvement in amino acid transport in mammary gland found support from the increase in GGT activity at the onset of lactation (Puente et al., 1982). Also, a correlation between GGT activity and changes in the arteriovenous differences of amino acids across the mammary gland under different experimental conditions has been observed (Vina et al., 1981a, 1986, 1989). Rekha and Kansal (1996a) showed that the uptake of amino acids by mouse mammary tissue explants having its GGT activity inhibited, by affinity labelling with 6-diazo-5-oxo-L-norleucine, was comparable with the uptake by the untreated mammary tissue explants. Hence, the gamma-glutamyl cycle is not involved in the transport of amino acid by mammary gland.

REGULATION OF AMINO ACID TRANSPORT BY LACTOGENIC HORMONES

Lactogenic hormones are responsible for the changes in mammary tissue whereby the mammary alveolar cells acquire the ability to secrete milk (Roh et al., 1994). The initiation of milk protein synthesis in mammary gland explants form mid-pregnant mice can be accomplish by incubation with insulin, cortisol and prolactin (Anderson and Rillema, 1976). The stimulation of lactogenesis in the mammary gland is a complex process involving the synergic action of a number of hormones such as insulin, glucocorticoids and prolactin. The provision of amino acids to the mammary cells may be a critical factor in stimulating protein anabolic processes by lactogenic hormones. Vina et al. (1981b) found that lactating rats treated with bromocryptine exhibited decreased arteriovenous concentration differences across the mammary gland of all amino acids except aspartate, glutamate and valine. Normal arteriovenous differences for most amino acids were restored by administering exogenous prolactin, suggesting that bromocryptine was exerting its action by inhibiting prolactin secretion from the pituitary. Shennan et al. (1997) have shown that mammary tissue from rat treated with bromocryptine exhibits a lower capacity for α-aminoisobutyric acid (AIB) uptake via both MeAIB and BCH-sensitive pathways compared with tissue taken from untreated animals. Sharma and Kansal (1999) studied the effect of lactogenic hormones (insulin, cortisol and prolactin) on the transport of L-alanine in cultured mammary tissue explants taken from pregnant mice. They showed that the A and L transport systems are upregulated by lactogenic hormones. Also, insulin alone upregulates systems A and L to some extent. Inclusion of all three lactogenic hormones increased system A and L activity 7 and 3 · 5 fold, respectively (table 3). The effect of insulin on the transport system A was confirmed by Rehan et al. (2000) for the uptake of glycine. The stimulation of amino acid uptake by mouse mammary gland cells is a consequence of increasing the V_{max} of transport with no change in the K_m. The action of the hormone has a latent period of 6 h and requires ongoing mRNA and protein synthesis (Rillema et al., 1992). The BCl^--independent system is

Table 3. Effect of hormones on L-alanine uptake by pregnant mouse mammary gland explants (mean values ±SEM, n=8, nmol min^{-1} g^{-1} cell)

Hormones	System A	BCl^--dependent	BCl^--independent	System L
None	0.62 ± 0.14^a	0	4.20 ± 0.09^a	0.64 ± 0.05^a
Insulin	1.57 ± 0.28^b	0	4.49 ± 0.29^a	1.23 ± 0.10^b
Insulin and cortisol	2.29 ± 0.29^b	0	4.27 ± 0.32^a	1.56 ± 0.03^b
Insulin, cortisol and prolaction	4.80 ± 0.22^c	0	6.78 ± 0.07^b	2.72 ± 0.33^c

Mammary gland explants from the pregnant mouse were cultured *in vitro* for 48 h in absence or presence of hormone(s). The uptakes of L-alanine (from 0.1 mM external L-alanine) were determined in media containing (1) Na^+ and Cl^-; (2) Na^+, Cl^- and 10mM-MeAIB; (3) Na^+ and 10mM-MeAIB ; (4) Cl^- but no Na^+; (5) Cl^- and 20mM-BCH but no Na^+. The system A was determined taking difference of L-alanine uptakes in media 1 and 2; The system BCl^--dependent was determined by taking difference of L-alanine uptakes in media 2 and 3; The BCl^--independent system was determined taking difference of L-alanine uptakes in media 3 and 4; The system L was determined taking difference of L-alanine uptakes in media 4 and 5. Values for the transport systems with different superscripts are significantly different ($p<0.01$). Adapted from Sharma and Kansal (1999).

Table 4. Effect of hormones on L-arginine uptake by pregnant mouse mammary gland explants[†] (value are nmol/5 min/g cells, mean ± SEM)

Hormones	Broad specificity system[‡] (n=6)	y^+system[§] (n=14)
None	20.3 ± 4.3^a	5.7 ± 0.4^a
Insulin	ND	17.6 ± 0.7^b
Insulin and cortisol	ND	17.5 ± 0.4^b
Insulin, cortisol and prolactin	18.0 ± 4.4^a	29.4 ± 0.7^c

ND, not determined

[†] Mammary gland explants from pregnant mouse were cultured *in vitro* for 48 h in the absence or presence of hormone(s). The uptakes of L-arginine (from an external concentration of 0.1 mM-L-arginine) were determined in media (1) Na^+-free buffer, (2) Na^+-free buffer with 20 mM-leucine, (3) Na^+-free buffer with 20 mM each of L-leucine and L-arginine.

[‡] Difference between L-arginine uptake in media 1 and 2.

[§] Difference between L-arginine uptake in media 2 and 3

[a,b,c] Values in same column with different superscript letters were significantly different : ($p<0.01$). Adapted from Sharma and Kansal (2000).

also upregulated in pregnant mouse mammary tissue when cultured in the presence of insulin, cortisol and prolactin. Insulin and cortisol in the absence of prolactin produces no affect, suggesting that prolactin is involved in the upregulation of the BCl^--independent system (table 3). The BCl^--dependent activity was not detected in pregnant mammary tissue, and lactogenic hormones failed to induce it *in vitro* (Sharma and Kansal, 1999). The mechanism for the induction of the BCl^--dependent system at the onset of lactation remains unclear. Prolactin also appears to regulate the transport of cationic amino acids since bromocryptine treatment of rats reduced the arteriovenous concentration difference of arginine and lysine across the mammary gland (Vina et al., 1981b). Sharma and Kansal (2000) showed that the y^+ system for the uptake of amino acids is upregulated by lactogenic hormones. They observed several fold increase in the transport of L-arginine via the y^+ system in pregnant mouse mammary tissue explants cultured in the presence of insulin, cortisol and prolactin. Insulin alone also increased the y^+ system activity approximately 2 fold. Lactogenic hormones (table 4) do not regulate the transport of cationic amino acids via broad specificity system y^+L. The high affinity Na^+-dependent anionic amino acid transport system is not regulated by prolactin, given that aspartate and glutamate extraction by mammary gland was unaffected by bromocryptine treatment of rats (Vina et al., 1981b). Furthermore, the transport of L-glutamate remained unaffected in pregnant mouse mammary tissue explants when cultured *in vitro* in the presence of lactogenic hormones, insulin, cortisol and prolactin (Kansal et al., 2000). The system T, specific for the transport of aromatic amino acids, is also not regulated by lactogenic hormones (Rekha, 1998). Milk accumulation appears to affect the entry of nutrients into mammary cells. Premature weaning decreases arterio-venous amino acid concentration differences across the rat mammary gland (Vina et al., 1981c). The transport systems A and L are down regulated by milk stasis (Shennan and McNeillie, 1994c). These

experiments were done on rats that were allowed to suckle their young but had the teats sealed on one side to prevent milk removal; therefore, these rats had both normal (i.e. sucked) and weaned glands each having the same blood flow and hormonal environment. Milk accumulation regulates the rate of milk secretion by an inhibitor in an autocrine fashion and decreases the number of prolactin receptors in the rat mammary gland (Hayden and Smith, 1981). The transduction system involved in the control of amino acid by milk stasis is not clear. Decreased amino acid transport may be a consequence of increased intramammary pressure and/or the accumulation of a milk-borne inhibitor The rate of milk secretion is regulated by an inhibitor (Feedback inhibitor of lactation) in an autocrine fashion.

CONCLUSIONS

The presence of nine transport systems has been established in the lactating mammary gland. These systems have been characterized with respect to their substrate specificity, affinity, kinetics and ion-dependence. Neutral amino acids are transported across the plasma membrane of lactating mammary gland via four Na^+-dependent systems (A, β, BCl^--dependent and BCl^--independent), which are coupled to sodium transmembrane gradient. The Na^+-independent systems that transport neutral amino acids are L, T and y^+L. The BCl^--dependent, BCl^--independent and y^+L are broad specificity systems that interact with both neutral and cationic amino acids. System T has specificity limited only to aromatic amino acids. The A system has high affinity for small chain neutral amino acids, while system L has greater affinity for long or branched chain aliphatic and aromatic amino acids. The uptake of anionic amino acids is mediated by a Na^+-dependent transport system X_{AG}^-. The transport of cationic amino acids is largely mediated by Na^+-independent system y^+ with some contribution by broad specificity systems. Our understanding is steadily improving about the regulation of transport systems by intracellular amino acid levels and by lactogenic hormones. At least four transport systems (A, L, y^+ and BCl^--independent) are upregulated by lactogenic hormones, at the onset of lactation. Future studies should focus (i) how precisely the transport systems interact to maintain intracellular pool of amino acids and ii) transduction systems involved in the hormonal control of nutrient transport, and induction and maintenance of lactation.

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