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The mechanism of L-glutamate transport by lactating rat mammary tissue

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Abstract

The transport of L-glutamate by lactating rat mammary gland has been examined using both tissue explants and a perfused mammary preparation. L-Glutamate uptake by mammary tissue explants was predominantly via a Na^+-dependent pathway: Li^+, choline$^+$ and $NMDG^+$ could not substitute for Na^+. L-Glutamate efflux from preloaded explants was also influenced by the transmembrane Na^+-gradient. These results are consistent with (Na^+-glutamate) cotransport. The Na^+-dependent system for L-glutamate transport in tissue explants was saturable (K_m = 112.5 ± 19.7 μM; V_{max} = 71.3 ± 10.4 nmol/min per g cells) and selective for anionic amino acids. Thus, D- and L-aspartate were high affinity inhibitors of L-glutamate uptake whereas neutral amino acids were relatively ineffective. D-Aspartate inhibited L-glutamate uptake in a competitive fashion. L-Glutamate uptake by the perfused mammary gland was (a) Na^+-dependent (b) saturable (K_m = 18.1 ± 4.9 μM; V_{max} = 40.3 ± 3.7 nmol/min per g tissue) and (c) selective for anionic amino acids. The results suggest that the (Na^+-glutamate) cotransporter is situated in the blood-facing aspect of the mammary epithelium.

Keywords: Glutamate transport; Mammary gland; Lactation

1. Introduction

L-Glutamate transport has been extensively studied in a large variety of cells and tissues. It appears that there are several systems which are capable of transporting this anionic amino acid across the plasma membranes of mammalian cells. Firstly, a high affinity (K_m 1–290 μM), Na^+-dependent mechanism, designated X^-_{AG} [1] has been described. This system plays an important role in terminating neurotransmission by removing L-glutamate from the synaptic cleft [2,3]. System X^-_{AG} also plays a role in the transport of L-glutamate in tissues such as the intestine, kidney, liver and placenta [3]. It appears that this mechanism operates by cotransporting two Na^+ ions with one glutamate in exchange for one K^+ and one OH^- [4,5]. System X^-_{AG} is characterised by being able to discriminate between the optical isomers of glutamate but not those of aspartate. Recently, several of the high-affinity Na^+-dependent glutamate transporters have been cloned and their primary amino acid sequences determined [6–8]. Secondly, a low affinity (K_m 2–4 mM), Na^+-dependent L-glutamate transporter has been identified in the intestine [9]. Interestingly, the low affinity pathway appears to have a substrate specificity similar to that of system X^-_{AG}. Thirdly, a Na^+-independent system, named x^-_C, is capable of transporting L-glutamate and cystine [1,10]. This transport mechanism may be important for regulating the levels of intracellular glutathione, a glutamate containing peptide, which is involved in protecting cells from oxidative stress.

Whilst the transport of L-glutamate has been thoroughly studied in kidney, intestine, placenta, liver and neuronal tissue other tissues have been overlooked. In this connection the transport of L-glutamate, and other anionic amino acids, by the lactating mammary gland has not received the attention it deserves. The lactating mammary gland has a high demand for glutamate in order to satisfy its demand for protein synthesis (for a review see [11]). Indeed, glutamate is the most abundant amino acid in milk protein accounting for 12% of the total amino acid residues in α S1 casein of bovine milk [12]. The mammary gland is able to generate a large arteriovenous L-glutamate concentration difference suggesting that mammary secretory cells possess a system(s) for transporting L-glutamate [13–16].

In view of the paucity of information relating to the

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mechanism of mammary tissue L-glutamate transport we have designed experiments to examine the transport of this anionic amino acid by lactating rat mammary tissue using both tissue explants and the perfused mammary gland.

2. Materials and methods

2.1. Animals

Lactating Wistar rats, 10–15 days post partum, which were suckling 8–10 pups were used throughout this study. The animals were maintained on a 12 h light–12 h dark cycle and allowed free access to water and chow.

2.2. Preparation of mammary tissue explants

Mammary tissue explants were prepared according to the method described by Shennan [17]. Rats were lightly anaesthetized with ether and then killed by cervical dislocation. The abdominal mammary glands were immediately removed and placed in a buffer similar in composition to that required for the glutamate flux studies (see figure legends for details). The tissue was finely chopped to produce explants weighing between 0.5 and 2 mg. Care was taken to remove as much connective tissue as possible. The tissue explants were rinsed repeatedly with buffer prior to the start of the experiment.

2.3. Measurement of L-glutamate transport in mammary tissue explants

2.3.1. L-Glutamate uptake

L-Glutamate uptake, using L-[^{3}H]glutamate as tracer was assayed at 37°C according to the method described in detail by Shennan [17]. Mammary tissue explants were incubated at 37°C in the appropriate buffer for at least 20 min prior to the addition of L-[^{3}H]glutamate. Following the addition of the radiotracer (at a final concentration of 0.25–0.5 μCi/ml) the tissue was removed at pre-determined intervals and immediately washed with 4 ml of an ice-cold solution similar in composition to the incubation medium except that it did not contain any isotope. The tissue explants were lightly blotted and placed in pre-weighed tubes. After the tissue weight had been determined, 4 ml of 10% trichloroacetic acid (TCA) was added. The tissue explants were left in the TCA f or at least 16 h. Afterwards the tubes were spun at 13 000 × g for 2 min. 1 ml of the resulting supernatants was removed and prepared for liquid scintillation counting. Aliquots of the incubation media were also counted.

L-Glutamate uptake was corrected for the amount of L-[^{3}H]glutamate associated with the extracellular space. Thus, the amount of [^{3}H]sucrose trapped in the tissue extracellular space was estimated in parallel experiments. The sucrose space was calculated from the ratio of [^{3}H]sucrose counts per g of tissue to [^{3}H]sucrose counts per ml of incubation medium.

The uptake of L-glutamate by tissue explants was calculated according to Eq. (1):

$$\left[\text{L} - [\text{glutamate}]_c\right. = \frac{[\text{L} - \text{glutamate}]_t - ([\text{L} - \text{glutamate}]_m \cdot F)}{1 - F} \quad (1)$$

where [L-glutamate]$_c$ is the cellular concentration of L-glutamate in nmol/g of cells, [L-glutamate]$_t$ is the total tissue concentration of L-glutamate in nmol/g of tissue wet weight, [L-glutamate]$_m$ is the concentration of L-glutamate in the incubation medium in nmol/ml and F is the sucrose space.

2.3.2. L-Glutamate efflux

L-Glutamate efflux, using L-[^{3}H]glutamate as tracer, was assayed according to the method described by Shennan et al. [18]. Mammary tissue explants were loaded with radiolabelled L-glutamate by incubation at 20°C for 30–40 min in a medium containing (mM) 0.001 glutamate, 135 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 1 amino(oxy)acetic acid, 10 glucose and 20 Tris-BES, pH 7.4 (+2–4 μCi L-[^{3}H]glutamate/ml). Following the loading period the tissue was transferred through a series of tubes containing 2.0 ml of radioactive free solutions. At the end of the efflux assay the tissue was allowed to stand in 4.0 ml of a 10% TCA solution for at least 16 h. This tube was spun at 13 000 × g for 2 min. 1 ml of the supernatant was removed and prepared for counting. The fractional efflux (min^{-1}) of L-glutamate from the tissue explants was calculated as previously described [18].

2.4. Perfusion of mammary tissue

The technique for the in situ, non-recirculating perfusion of inguinal/abdominal rat mammary tissue was essentially as described by Clegg and Calvert [19]. Anaesthesia was induced and maintained throughout by an i.p. injection of a sodium pentobarbitone solution (40 mg/kg of body wt.). The mammary gland was perfused via the superficial epigastric vessels. In control experiments the perfusate contained (mM) 118 NaCl, 4 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 1 NaH_2PO_4, 10 glucose and 25 $NaHCO_3$. The buffer was gassed to pH 7.4 with 95% O_2:5% CO_2. The perfusate was supplemented with 5% (w/v) bovine serum albumin (BSA). When the Na^+-dependence of L-glutamate transport was examined NaCl and NaH_2PO_4 were respectively replaced with an equimolar amount of *N*-methyl-D-glucamine ($NMDG^+$)-Cl and KH_2PO_4. In addition, $NaHCO_3$ was replaced in both the control and Na^+-free perfusates with 20 mM Tris-Hepes, pH 7.4. In these experiments the perfusates were gassed with 100% O_2.

2.5. Measurement of L-glutamate transport by the perfused mammary gland

The transport of L-glutamate, using L-[^{3}H]glutamate as tracer, by the perfused mammary gland was measured using a rapid, paired tracer dilution technique first described by Yudilevich et al. [20]. A bolus containing L-[^{3}H]glutamate and [^{14}C]sucrose (the extracellular marker) was injected close arterially (60 μl in < 2 s) and 20 consecutive venous outflow samples were collected. The uptake (U) of L-glutamate for each collection period was calculated according to Eq. (2):

$$U(\%) = 1 - (R_t/R_i) \quad (2)$$

where R_t is the ratio of L-[^{3}H]glutamate/[^{14}C]sucrose in the venous outflow at time t after the bolus injection and R_i is the ratio of L-[^{3}H]glutamate/[^{14}C]sucrose in the injectate. To standardise L-glutamate transport between each experiment we expressed the uptakes as a clearance (i.e., the volume of perfusate notionally cleared of L-[^{3}H]glutamate). The clearances were calculated by multiplying the uptakes (U) for each collection period by the perfusate flow rates (ml/min per g of tissue wet wt.).

In the experiments where the perfusates contained a known amount of unlabelled L-glutamate it was possible to calculate the unidirectional uptake (V_t) from the maximum uptake (U_{max}) according to Eq. (3):

$$V_t = -\ln(1 - U_{max}) \times F \times C \quad (3)$$

where F is the perfusate flow and C is the perfusate concentration of unlabelled L-glutamate.

2.5.1. Statistics

Where appropriate, values are expressed as mean ± S.E.M. Differences were assessed by Student's paired or unpaired t-tests and were considered significant when $P < 0.05$.

2.5.2. Chemicals

L-[^{3}H]Glutamate, [^{3}H]sucrose and [^{14}C]sucrose were obtained from Amersham International, Aylesbury, Bucks., UK. All other chemicals including the unlabelled amino acids were purchased from Sigma, Poole, Dorset, UK.

3. Results

3.1. L-Glutamate transport by mammary tissue explants

3.1.1. Cation dependency of L-glutamate uptake by mammary tissue explants

Preliminary experiments revealed that L-glutamate uptake (5 μM) by mammary tissue explants was linear for at least 3 min (Fig. 1, inset). Therefore, in subsequent experiments L-glutamate influx was measured after 2 min of incubation. In one experiment we found that L-glutamate uptake at 1 mM was linear for 2 min (results not shown). Fig. 1 illustates the effect of replacing extracellular Na^+ with either choline$^+$, NMDG$^+$ or Li^+ on L-glutamate uptake. Glutamate uptake was almost abolished when the incubation medium did not contain Na^+ ($P < 0.001$). It appears, therefore, that the predominant route for L-glutamate uptake into mammary tissue explants is a Na^+-dependent pathway.

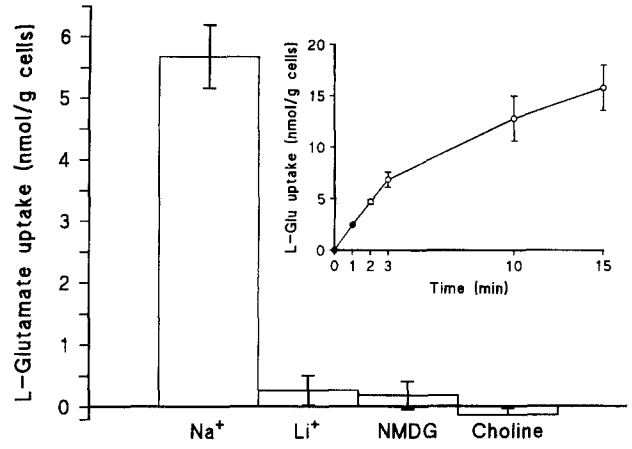

Fig. 1. The cation dependency of L-glutamate uptake by mammary tissue explants. Tissue was incubated in a medium containing (mM) 0.005 L-glutamate, 135 XCl (where X = Na^+, choline$^+$, Li^+ or NMDG$^+$), 5 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 10 glucose and 20 Tris-Mops, pH 7.4. L-Glutamate uptake was assayed at 37°C after 2 min of incubation. The inset shows the time-course of L-glutamate uptake by mammary tissue explants incubated in a Na^+ buffer. Results shown are means ± S.E.M. of 3 experiments.

3.1.2. Cation dependency of L-glutamate efflux from mammary tissue explants

If the Na^+-dependent component of L-glutamate uptake represented (Na^+-glutamate) cotransport it was predicted that L-glutamate efflux from mammary tissue explants should also display a dependency on the transmembrane Na^+-gradient. This prediction was based on the observation of Bouvier et al. [4] that the Na^+-dependent glutamate uptake carrier in glial cells can run backwards. Therefore, to test this prediction we studied the effect of reversing the Na^+-gradient on the efflux of L-glutamate from tissue explants which were preloaded with radiolabelled L-glutamate. To do this we measured glutamate efflux into a buffer containing Na^+ as the principal cation followed by one where Na^+ had been replaced by choline. The efflux rate constant of L-glutamate was increased from 0.0145 ± 0.0003 min^{-1} to 0.0299 ± 0.0017 min^{-1} (± S.E.M., $n = 6$, $P < 0.001$) by reversing the Na^+ gradient in this manner.

3.1.3. Kinetic parameters of L-glutamate uptake by mammary tissue explants

The kinetic parameters (i.e., K_m and V_{max}) of L-glutamate uptake by mammary tissue explants were examined. In this set of experiments the L-glutamate concentration in

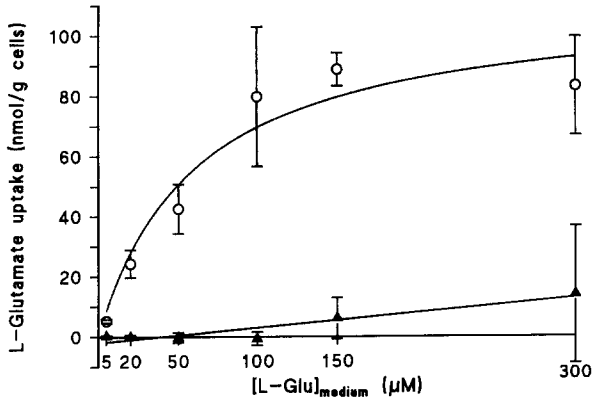

Fig. 2. The L-glutamate concentration dependence of L-glutamate uptake by mammary tissue explants in the presence (○) and absence (▲) of Na^+. Tissue was incubated in a buffer containing (mM) 0.005–0.3 L-glutamate, 135 XCl (where X = Na^+ or choline$^+$), 5 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 10 glucose and 20 Tris-Mops, pH 7.4. L-Glutamate uptake was assayed at 37°C after 2 min of incubation. The points shown are the means ± S.E.M. of 5 experiments.

the incubation medium was varied over the range 5–300 μM in the presence and absence of Na^+ (choline replacement). It is clear from Fig. 2 that L-glutamate uptake, in the presence of Na^+, was saturable with respect to the extracellular L-glutamate concentration. In contrast, L-glutamate uptake in the absence of extracellular Na^+ was substantially reduced and was not saturable over the concentration range tested. A Hanes-Woolf plot of the Na^+-dependent moiety of uptake suggested that there was a single system for L-glutamate uptake. The calculated K_m and V_{max} was respectively 112.5 ± 19.7 μM and 71.3 ± 10.4 nmol/min per g of cells (± S.E.M., $n = 5$).

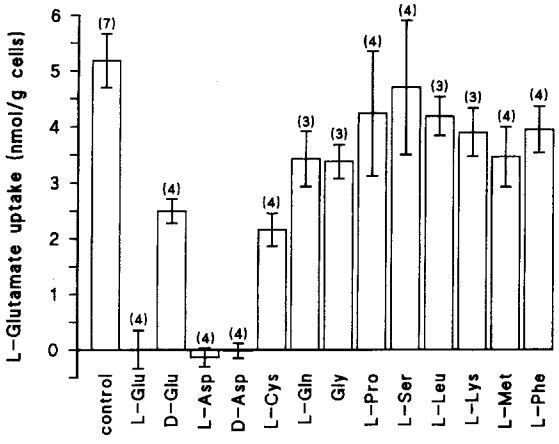

Fig. 3. The effect of amino acids on L-glutamate uptake by mammary tissue explants. Tissue was incubated in a buffer containing (mM) 0.005 L-glutamate, 135 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 10 glucose and 20 Tris-Mops, pH 7.4. When required, the test amino acids were present at 5mM. L-Glutamate uptake was assayed at 37°C after 2 min of incubation. The numbers in parentheses represent the number of experiments performed with each amino acid. Results shown are means ± S.E.M.

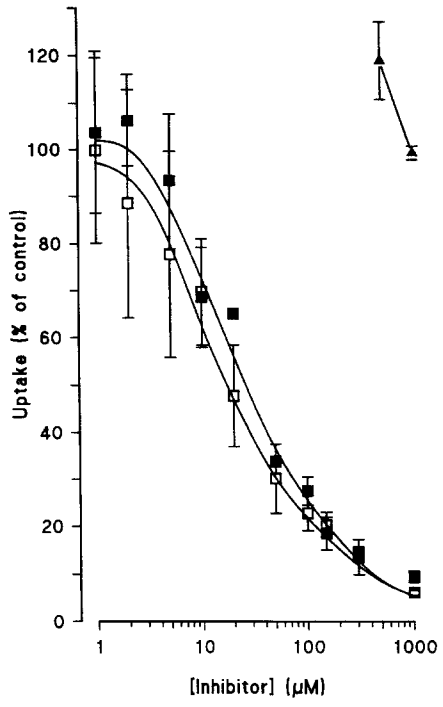

Fig. 4. The concentration dependence of inhibition by anionic amino acids of L-glutamate uptake by mammary tissue explants. The points denoted (■), (□), and (▲), respectively, represent the addition of varying concentrations of L-aspartate, D-aspartate, and D-glutamate. The incubation conditions were the same as those described in Fig 3. The points shown are the means ± S.E.M of 3 experiments.

3.1.4. Amino acid specificity of the L-glutamate carrier in mammary tissue explants

We next studied the substrate specificity of the Na^+-dependent carrier by examining the effect of adding a variety of unlabelled amino acids (at 5 mM) to the incubation medium on glutamate uptake (at 5 μM). It is evident from Fig. 3 that L-glutamate uptake was markedly inhibited by D- and L-aspartate and less so by D-glutamate. Fig. 3 also shows that L-glutamate uptake was relatively insensitive to neutral amino acids and to L-lysine. These results suggested to us that the L-glutamate carrier was selective for anionic amino acids. The concentration dependence of inhibition by the anionic amino acids was then examined. The IC_{50} of inhibition by D- and L-aspartate, calculated from the data shown in Fig. 4 was 15.9 ± 8.8 μM and 40.6 ± 0.7 μM, respectively. The IC_{50} of inhibition by D-glutamate was greater than 1 mM. The inhibition of L-glutamate uptake by D-aspartate was found to be of a competitive nature, thus, the presence of 20 μM D-aspartate in the incubation medium increased the K_m of L-glutamate uptake from 112 ± 19.7 μM to 408.6 ± 103.9 μM (± S.E.M., $n = 3$, $P < 0.05$). In contrast the presence of aspartate did not significantly change the V_{max} (71.3 ± 10.4 vs. 85.8 ± 18.9 nmol/min per g of cells, respectively, in the absence and presence of D-aspartate).

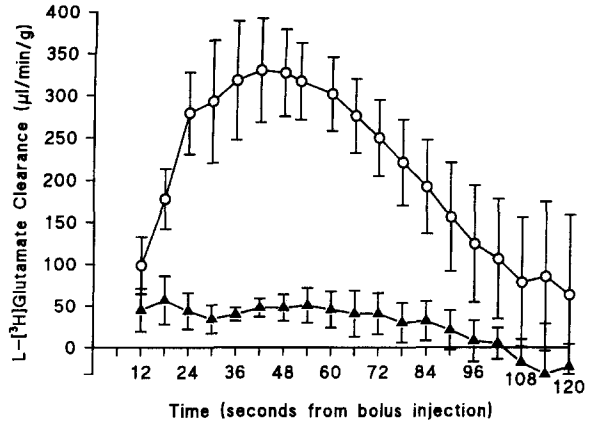

Fig. 5. The clearance profile of L-glutamate by the perfused mammary gland in the presence (○) and absence (▲) of Na^+ in the perfusate. The gland was perfused with a buffer containing (mM) 118 XCl (where $X = Na^+$ or $NMDG^+$), 4 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 1 KH_2PO_4, 10 glucose and 20 Tris-Hepes, pH 7.4. The perfusate also contained BSA (5%, w/v) and was gassed with 100% O_2. The points shown are the means ± S.E.M. of 5 experiments.

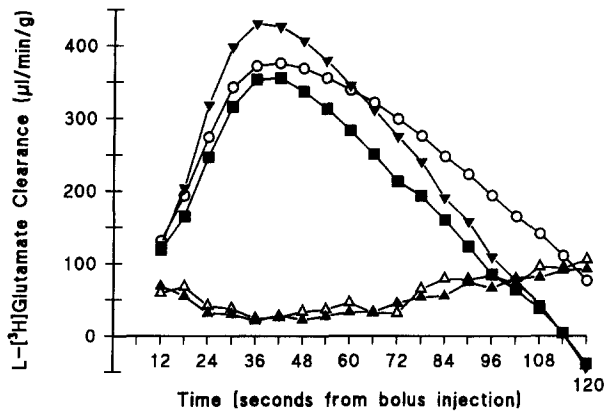

Fig. 6. The effect of unlabelled D-aspartate (△), L-aspartate (▲), D-glutamate (▼) and L-leucine (■) on the clearance of L-glutamate by the perfused mammary gland. Each amino acid was added to the injectate at a concentration of 50 mM. The clearance in the absence of amino acids (i.e., the control) is shown by the open circles. The perfusate contained (mM) 118 NaCl, 4 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 1 NaH_2PO_4, 10 glucose and 25 $NaHCO_3$. The perfusate also contained 5% BSA (w/v) and was gassed to pH 7.4 with 95% O_2 / 5% CO_2. The points shown are the means of 3 experiments. For the sake of clarity error bars have been omitted.

3.2. L-Glutamate transport by the perfused mammary preparation

3.2.1. Na^+-dependence of transport by the perfused mammary preparation

Fig. 5 shows the clearance profile of L-[^{3}H]glutamate by the perfused gland in the presence and absence of Na^+. In this set of experiments the perfusate and injectate did not contain any unlabelled L-glutamate: this was to maximize the extraction of L-[^{3}H]glutamate by the mammary gland. In the presence of extacellular Na^+, the time course of L-[^{3}H]glutamate clearance consisted of a rising phase followed by a rapid fall off. The rising phase is governed by the unidirectional uptake of radiolabelled L-glutamate. The presence of a rising phase suggests that there may be a significant portion of the perfusate which bypasses capillaries supplying the secretory acini (i.e. an A-V shunt). The fall off is probably due to tracer efflux from the tissue back into the perfusate. It is evident from Fig. 5 that the clearance of tracer L-glutamate is Na^+-dependent, thus, replacing Na^+ with $NMDG^+$ resulted in a marked fall in the clearance.

3.2.2. Substrate specificity of L-glutamate transport by the perfused mammary preparation

To characterise the transport of L-[^{3}H]glutamate by the perfused gland with respect to substrate specificity we studied the effect of a range of amino acids on the clearance of L-[^{3}H]glutamate. On the basis of the explant studies we examined the effect of D-glutamate, D- and L-aspartate and L-leucine. In this series of experiments the 'test' amino acids were co-injected with the radiotracers. Thus, the bolus contained the amino acids at a concentration of 50 mM. Although this appears to be a high concentration of the putative substrates it must be borne in mind that the concentration at the site of transport would have been lower owing to the diluting effect of the perfusate. It is clear from Fig. 6 that the clearance of L-[^{3}H]glutamate was reduced by aspartate (both the D and L isomers) but not by D-glutamate or L-leucine.

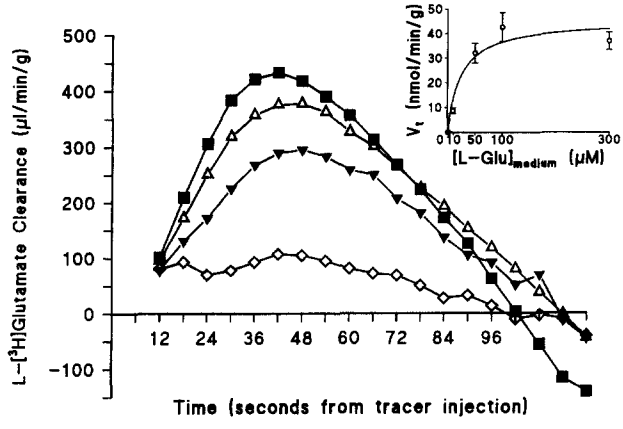

Fig. 7. The concentration dependence of L-glutamate clearance by the perfused mammary gland. The perfusate contained varying concentrations of unlabelled L-glutamate; (■) 10 µM, (△) 50 µM, (▼) 100 µM, (◇) 300 µM. The perfusate also contained (mM) 118 NaCl, 4 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 1 KH_2PO_4, 10 glucose and 25 $NaHCO_3$. The perfusate was supplemented with 5% BSA (w/v) and was gassed to pH 7.4 with 95% O_2 /5% CO_2. The points shown are the means of 3 experiments. For the sake of clarity error bars have been omitted. The inset shows the unidirectional influx (V_t) plotted as a function of the L-glutamate concentration.

3.2.3. Kinetics of L-glutamate transport by the perfused mammary preparation

The kinetics parameters (i.e., the K_m and V_{max}) of L-glutamate transport by the perfused lactating mammary gland were examined. Unlabelled L-glutamate, at known concentrations, was added to both the perfusate and injectate and the clearance of L-[^{3}H]glutamate was measured for each concentration (Fig. 7). Apparently, the clearance of radiolabelled glutamate decreased as the concentration of unlabelled L-glutamate increased: this is consistent with the presence of carrier mediated transport. The unidirectional uptake (V_t) of glutamate, using the U_{max} values, was calculated according to equation 3. V_t was plotted as a function of the perfusate L-glutamate concentration (Fig. 7). It is clear that L-glutamate uptake by the perfused gland, like that of tissue explants, displays saturation kinetics. Plotting the data shown in Fig. 7 as a Hanes-Woolf plot revealed that the K_m and V_{max} of transport were, respectively, 18.1 ± 4.9 μM and 40.3 ± 3.7 nmol/min per g of tissue (± S.E.M., $n = 3$).

4. Discussion

The experiments described in this report were conducted to investigate the mechanism of L-glutamate transport by lactating rat mammary tissue. This was done because there is no information about L-glutamate transport in this tissue despite the importance of this anionic amino acid to mammary gland metabolism. We employed two experimental systems to measure L-glutamate transport by rat mammary tissue, namely tissue explants and the perfused mammary gland. We used the data generated from the explant experiments to design experiments using the technically demanding perfused mammary preparation thus maximising our efforts.

It is apparent that the major pathway for L-glutamate uptake by mammary tissue is a Na^+-dependent system. This taken together with the finding that the efflux of L-glutamate from mammary tissue explants can be increased by reversing the transmembrane Na^+-gradient suggests that the mechanism of transport is a (Na^+-glutamate) cotransporter. This cotransporter is a saturable system which appears to prefer anionic amino acids as substrates. The mammary glutamate carrier was inhibited by D- and L-aspartate with similar affinity but was more sensitive to L-glutamate than D-glutamate. Indeed, the finding that the IC_{50} of inhibition of L-glutamate transport by the isomers of aspartate was lower than the K_m of glutamate transport suggests that the carrier may have a higher affinity for aspartate. This pattern of selectivity leads us to suggest that the Na^+-dependent L-glutamate carrier in mammary tissue may be system X^-_{AG} which has been described elsewhere. System X^-_{AG} couples the transport of Na^+ and L-glutamate to the efflux of K^+ and OH^-. The experimental systems we used did not readily allow us to examine the role of K^+ in the transport of L-glutamate by the mammary gland.

L-Glutamate uptake by the perfused lactating mammary gland had similar characteristics to that of L-glutamate transport in explants. Thus, L-glutamate extraction by the perfused gland was via a high affinity, Na^+-dependent system which preferred anionic amino acids as substrates. An analysis of the kinetic data suggested that there is only one system for L-glutamate transport in mammary tissue explants. Therefore, it would appear that the system identified in explants is situated in the blood-facing aspect of the mammary epithelium. However, we cannot rule out the presence of a Na^+-dependent system in the apical aspect of the epithelium which has kinetic characteristics identical to that of the carrier in the basolateral membranes. A Na^+-dependent L-glutamate carrier situated in the basolateral membrane of mammary secretory cells would be ideally situated to generate the large intra- to extracellular L-glutamate concentration gradient across the plasma membranes of mammary secretory cells [18]. Interestingly, despite this large gradient it seems that mammary tissue, unlike other tissues [21,22], does not use L-glutamate to volume regulate following swelling. Thus, Shennan et al. [18] found that the efflux of L-glutamate, unlike that of taurine and glycine, was unaffected by a hyposmotic challenge and hence cell-swelling.

Although the characteristics of L-glutamate transport by the perfused mammary preparation were similar to that found with explants there was an interesting difference in the kinetic parameters. The K_m for L-glutamate uptake by explants and the perfused gland was respectively 112.5 and 18.1 μM. We believe that the difference reflects the presence of larger unstirred water layers in the tissue explants compared to that found in the perfused gland. Such layers would mean that the concentration of substrate at the site of transport could be significantly less than that of the bulk phase and would lead to an overestimation of the K_m in tissue explants [23]. However, the K_m of L-glutamate transport obtained by both preparations falls within the range previously reported for system X^-_{AG}.

The lactating rat mammary gland is capable of generating a large arterio-venous glutamine concentration gradient [16]. Indeed, in the rat, the mammary arterio-venous glutamine gradient is larger than that for glutamate. Glutamine is an important source of glutamate in a number of cell types. However, the present finding that mammary cells express a glutamate carrier suggests that rat mammary cells do not have to completely rely on glutamine as a source of intracellular glutamate.

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