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Biochimica et Biophysica Acta 1421 (1999) 340–346

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

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The regulation of Na^+-dependent anionic amino acid transport by the rat mammary gland

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Received 10 June 1999; received in revised form 6 August 1999; accepted 12 August 1999

Abstract

The regulation of anionic amino acid transport, using radiolabelled D-aspartate as a tracer, by rat mammary tissue explants has been examined. Na^+-dependent D-aspartate uptake by mammary tissue increased between late pregnancy and early lactation and again at peak lactation but thereafter declined during late lactation. In contrast, the Na^+-independent component of D-aspartate uptake by mammary explants did not change significantly with the physiological state of the donor animals. Premature weaning of rats during peak lactation markedly decreased Na^+-dependent D-aspartate uptake by mammary tissue. In addition, premature weaning also reduced the effect of reversing the *trans*-membrane Na^+-gradient on the fractional loss of D-aspartate from mammary tissue explants. Unilateral weaning of rats during peak lactation revealed that milk accumulation per se reduced the Na^+-dependent moiety of D-aspartate uptake by mammary tissue suggesting that the transport of anionic amino acids is regulated to match supply with demand. Treating lactating rats with bromocryptine reduced D-aspartate uptake by mammary tissue explants suggesting that the transport of anionic amino acids by the rat mammary gland is regulated by prolactin.

Keywords: Lactation; Amino acid; Transport

1. Introduction

During lactation, the mammary gland has a large demand for amino acids to meet the needs of milk protein synthesis: this is reflected in large arteriovenous amino acid concentration gradients across the gland [1]. Amino acids are extracted from the circulation by transport systems, each having distinct ionic and substrate specificities, situated in the basolateral membranes of the mammary secretory cells (for a review see [2]). Depending on species, neutral amino acids are transported via systems resembling A, ASC, L and T [3–8], anionic amino acids utilise a Na^+-dependent mechanism [9,10] and taurine is taken up via system β [11]. In addition, lactating mammary tissue expresses a system(s) which accept cationic amino acids as substrates [12–14] and a volume-activated amino acid transport mechanism [15]. Although a picture is emerging of the discrete transport systems present in mammary tissue, very little is known about the control of individual mechanisms. A thorough knowledge of the regulation of mammary tissue amino acid transport mechanisms is required if we are to fully understand the process of milk protein synthesis.

In this study we have investigated the control of anionic amino acid transport by the rat mammary

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0005-2736/99/$ – see front matter
PII: S0005-2736(99)00140-6

gland. Anionic amino acid transport across the basolateral pole of the lactating rat mammary epithelium is dependent upon Na^+ in a fashion consistent with (Na^+-anionic amino acid) cotransport [9,10] and appears to resemble system X^-_{AG} [16]. In particular, we have examined the development of Na^+-dependent anionic amino acid transport in rat mammary tissue. Thus, we have studied the transport of D-aspartate, a non-metabolised amino acid, by mammary tissue isolated from rats during pregnancy and at different stages of lactation. It has been established that D-aspartate is transported by the high affinity, Na^+-dependent system which accepts L-glutamate and L-aspartate as substrates [10]. Moreover, it appears that the Na^+-dependent mechanism is the predominant, if not only, route available for D-aspartate transport into mammary cells [10]. In addition, we have investigated the regulation of mammary tissue D-aspartate transport by prolactin and milk stasis.

2. Materials and methods

2.1. Animals

Wistar rats were used in this study. The animals were fed standard rat chow and allowed free access to water. The animals were housed at a constant temperature of 17°C under conditions of 12 h light-dark cycles. Mammary tissue explants, prepared as previously described [9], were isolated from late pregnant (day 17–18), early lactating (day 1–2), peak lactating (day 11–14) and late lactating animals (day 22). Lactating animals were suckling 8–10 pups.

2.2. Premature weaning of lactating rats

Lactating rats during peak lactation (day 11–14) were removed from their litters and mammary tissue explants were prepared 24 h later.

2.3. Unilateral weaning of lactating rats

Lactating rats during peak lactation (day 12–13) which were allowed to suckle their young had the teats on one side sealed with adhesive to prevent milk removal for 24 h prior to the preparation of mammary tissue explants.

2.4. Bromocryptine treatment of lactating rats

When the inhibition of prolactin secretion was required, lactating rats during peak lactation (day 12–13) were injected subcutaneously with bromocryptine (2-bromo-α-ergocryptine dissolved in 50% ethanol:50% saline). Each animal received 2 mg divided equally between two doses which were administered at 9:30 and 16:30 h the day prior to killing. The efficacy of the bromocryptine treatment was judged by measuring daily pup weight gain (a measure of milk production).

2.5. D-Aspartate uptake by mammary tissue explants

D-Aspartate uptake, using D-[^{3}H]aspartate as tracer, was measured according to the method described in detail by Millar et al. [10]. Briefly, tissue explants (each weighing 0.5–2.0 mg wet weight, 20–30 mg total) were incubated in 6 ml of buffer containing 0.005 mM D-aspartate, 135 mM XCl (where X = Na^+ or choline), 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgSO_4$, 10 mM glucose and 20 mM Tris-MOPS, pH 7.4 at 37°C for 20 min prior to the addition of radiolabelled D-aspartate (0.25–0.5 μCi/ml). Following the addition of isotope, mammary explants were removed at pre-determined intervals and immediately washed with 4 ml of an ice-cold buffer. The tissue explants were lightly blotted, weighed and then placed in 4 ml of distilled H_2O at least 16 h. A 1 ml portion of the extract was counted to determine the amount of radioactivity associated with the explants. The specific activity of radiolabelled D-aspartate in each incubation medium was determined by counting the radioactivity in 100 μl samples. The tissue extracellular space was estimated by measuring the distribution of [^{3}H]sucrose between the incubation medium and the tissue explants in parallel experiments. D-aspartate uptake was calculated according to:

$$[\text{D-aspartate}]_c = \frac{[\text{D-aspartate}]_t - ([\text{D-aspartate}]_m \cdot F)}{1-F} \quad (1)$$

where $[\text{D-aspartate}]_c$ is the cellular concentration of D-aspartate in nmol/g cell wet weight, $[\text{D-aspartate}]_t$ is the total tissue concentration of D-aspartate in nmol/g tissue wet weight, $[\text{D-aspartate}]_m$ is the con-

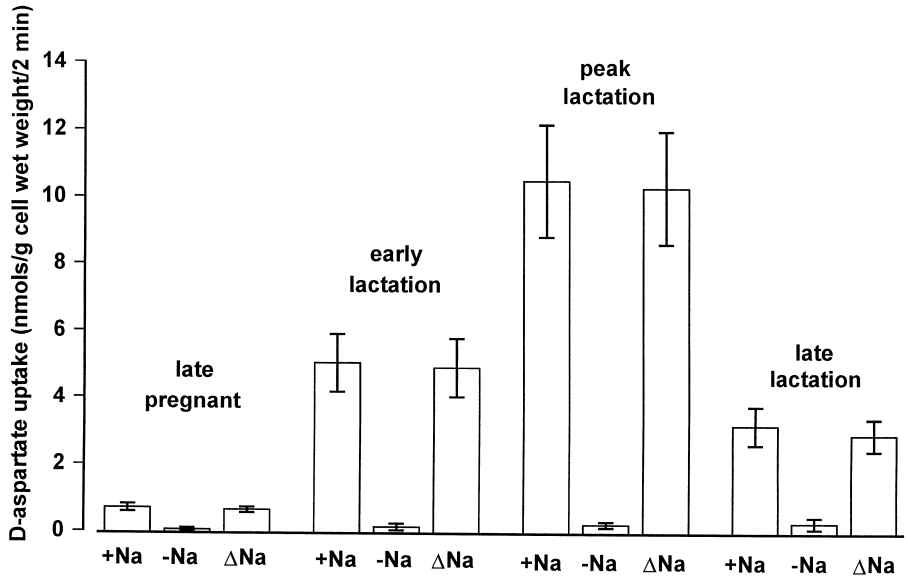

Fig. 1. D-Aspartate uptake by mammary tissue explants isolated from rats during late pregnancy and at various stages of pregnancy/lactation in the presence and absence of extracellular Na^+. Mammary explants were incubated in a medium containing 0.005 mM D-aspartate, 135 mM XCl (where $X = Na^+$ or choline), 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgSO_4$, 10 mM glucose and 20 mM Tris-MOPS, pH 7.4. Data represent the mean ± S.E.M. of three experiments (seven experiments when D-aspartate uptake by peak lactating tissue in the presence of Na^+ was measured).

centration of D-aspartate in the incubation medium in nmol/ml and F is the fractional sucrose space.

2.6. Taurine uptake by mammary tissue explants

Taurine uptake by mammary tissue was measured using [^{3}H]taurine as tracer. The incubation medium contained 0.001 mM taurine, 135 mM XCl (where $X = Na^+$ or choline), 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgSO_4$, 10 mM glucose and 20 mM Tris-MOPS, pH 7.4 (+1–2 μCi/ml [^{3}H]taurine). The method used to measure taurine uptake was essentially the same as that used to measure D-aspartate uptake with the main exception that taurine uptake was assayed after 30 min of incubation.

2.7. D-Aspartate efflux from mammary tissue explants

D-Aspartate efflux, using D-[^{3}H]aspartate as tracer, was measured according to the method described by Millar et al. [10]. Mammary tissue explants (4–8 mg wet weight) were loaded with radiolabelled D-aspartate by incubation at 20°C for 30–40 min in a buffer containing 135 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgSO_4$, 10 mM glucose and 20 mM Tris-MOPS, pH 7.4 (+2–4 μCi/ml of D-[^{3}H]aspartate). After the loading period the tissue explants were transferred through a series of tubes containing 2 ml of radioactive free solutions (composition given in the figure legends) after which the tissue was allowed to stand in 4 ml of distilled water for at least 16 h. The fractional efflux (min^{-1}) of D-aspartate from the tissue explants was calculated for each collection period according to:

$$\text{Fractional efflux} = \Delta X / \Delta t X_t \quad (2)$$

where ΔX represents D-aspartate released (dpm) during the interval Δt and X_t is the tissue D-aspartate content (dpm) at the start of each sampling period.

2.8. Materials

[^{3}H]Taurine and [^{3}H]D-aspartate were purchased from Amersham International UK. All other chemicals were obtained from Sigma, UK.

2.9. Statistics

Data are presented as mean ± S.E.M. Differences were assessed by Student's paired or unpaired t-test

as appropriate and were considered significant when $P < 0.05$.

3. Results

3.1. D-Aspartate uptake by mammary tissue at different stages of pregnancy/lactation

The transport of D-aspartate by mammary tissue taken from rats during late pregnancy and at different stages of lactation was examined. We established that D-aspartate uptake by mammary tissue explants isolated from pregnant and lactating rats was linear for at least 3 min (results not shown). Therefore, D-aspartate uptake by mammary tissue explants measured after 2 min of incubation was taken as an initial rate of uptake. Fig. 1 shows D-aspartate uptake by mammary explants in the presence and absence of extracellular Na^+. Na^+-dependent D-aspartate transport was relatively low in late pregnancy (0.643 ± 0.082 nmol/g cell wet weight/2 min) rising significantly ($P < 0.05$) at early lactation (4.898 ± 0.870 nmol/g cell wet weight/2 min) and again at peak lactation (10.240 ± 1.672 nmol/g cell wet weight/2 min; $P < 0.05$ vs early lactation). By late lactation, (Na^+-anionic amino acid) cotransport was 2.881 ± 0.479 nmol/g cell wet weight/2 min, a level significantly lower than that found during peak lactation ($P < 0.01$) but similar to that found in early lactation. It is evident from Fig. 1 that the Na^+-independent component of D-aspartate uptake did not change with the physiological state of the donor animals.

For comparison, we studied taurine transport by mammary tissue explants isolated from rats during early and peak lactation. Fig. 2 illustrates that (Na^+-taurine) cotransport decreased as lactation progressed, thus, Na^+-dependent taurine uptake by mammary tissue at day 3 and day 12–14 of lactation was 3.493 ± 0.364 and 1.155 ± 0.207 nmol/g cell wet weight/30 min, respectively ($P < 0.01$). In contrast, the Na^+-independent component of taurine uptake did not change during lactation.

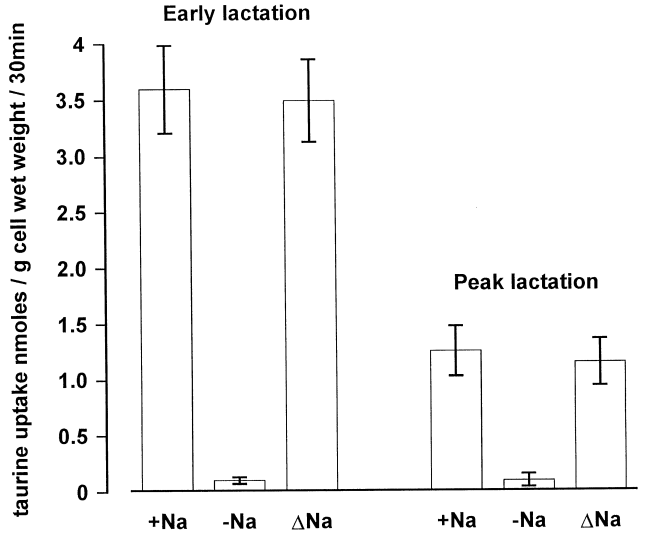

Fig. 2. Taurine uptake by mammary tissue explants isolated from rats during early (day 3) and peak (day 12–14) of lactation. Tissue was incubated in a medium containing 0.001 mM taurine, 135 mM XCl (where X = Na^+ or choline), 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgSO_4$, 10 mM glucose and 20 mM Tris-MOPS, pH 7.4. Data shown are means ± S.E.M. of four and three experiments, respectively using tissue from animals during early and peak lactation.

3.2. The effect of premature weaning on D-aspartate transport

It has previously been shown that premature weaning of lactating rats markedly reduces the arteriovenous concentration differences across the mammary gland for all amino acids including L-glutamate and L-aspartate [17]. On this basis, it was predicted that (Na^+-anionic amino acid) cotransport would be regulated by litter removal. To test this prediction we examined the uptake of D-aspartate by mammary tissue explants isolated from rats at peak lactation (day 12–13) which had been removed from their pups for 24 h. D-aspartate uptake by mammary tissue explants from prematurely weaned rats in the presence and absence of extracellular Na^+ was 1.643 ± 0.353 and 0.342 ± 0.068 nmol/g cell wet weight/2 min, respectively ($n = 3$). Thus, it is apparent that premature weaning reduced the Na^+-dependent moiety of D-aspartate uptake by 87% ($P < 0.001$) compared with mammary tissue taken from control animals at peak lactation (see above).

We also examined the effect of premature weaning on D-aspartate efflux from mammary tissue explants. D-aspartate efflux via the (Na^+-anionic amino acid) transport system can be increased by reversing the *trans*-membrane Na^+-gradient [10]. We studied the

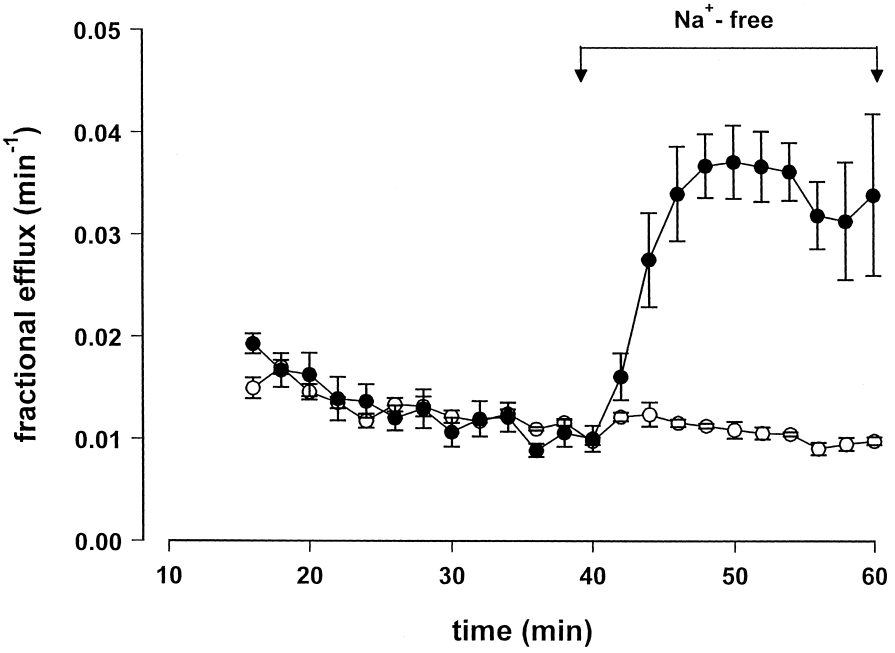

Fig. 3. The effect of reversing the Na^+-gradient on D-aspartate efflux from rat mammary explants isolated from peak lactating rats (●) and rats during peak lactation which had been removed from their litters for 24 h (○). Tissue explants were incubated first in a medium containing 135 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgSO_4$, 10 mM glucose and 20 mM Tris-MOPS, pH 7.4 followed by one of similar composition except that choline replaced the Na^+. Each point is the mean ± S.E.M. of three and four experiments, respectively using tissue from suckled and weaned animals.

effect of reversing the *trans*-membrane Na^+-gradient on D-aspartate efflux from tissue isolated from rats during peak lactation (i.e. controls) and from rats during peak lactation which had been prematurely weaned. Fig. 3 shows, in agreement with previous results from this laboratory [10], that reversing the Na^+-gradient markedly increased ($P < 0.01$) the efflux of D-aspartate from mammary explants isolated from the control animals, thus, the fractional release was increased (trough to peak) from 0.0100 ± 0.0012 (min^{-1}) to 0.0371 ± 0.0036 (min^{-1}). On the other hand, reversing the Na^+-gradient had no significant effect on the fractional release of D-aspartate from tissue taken from prematurely weaned rats.

The effect of premature weaning on D-aspartate uptake via the high affinity carrier could be a consequence of milk stasis and/or changes in the circulating levels of endocrine factors (e.g. prolactin). Therefore, to test whether milk accumulation within the lumen of the mammary gland affects D-aspartate transport per se we examined the effect of unilateral weaning [17]. Rats during peak lactation, which were allowed to suckle their young, had the teats on one side sealed with adhesive to prevent milk removal for 24 h. Thus, these rats had both control (i.e. suckled) and weaned glands each, presumably, having the same blood flow and hormonal environment [17]. The uptake of D-aspartate by mammary explants isolated from the weaned gland was reduced by approximately 78% compared to that by tissue taken from the suckled gland: D-aspartate uptake by mammary tissue explants isolated from the suckled and weaned glands was respectively 4.516 ± 0.665 and 0.984 ± 0.232 nmol/g cell wet weight/2 min ($n = 3$, $P < 0.05$).

3.3. The effect of bromocryptine treatment on D-aspartate uptake by mammary explants

The regulation of D-aspartate transport in mammary tissue by prolactin was examined by treating rats during peak lactation with bromocryptine. Daily pup weight gain before and after bromocryptine treatment was used to assess the inhibition of prolactin secretion. Daily litter weight gain 24–48 h and 0–24 h prior to treatment was found to be

17.23 ± 1.50 g and 17.60 ± 1.40 g, respectively. Litter weight gain 24 h after the start of bromocryptine administration was 6.97 ± 1.54 g suggesting that milk production was reduced. It was apparent that bromocryptine treatment, and hence a reduction in prolactin secretion, markedly inhibited D-aspartate uptake. Thus, D-aspartate uptake by mammary tissue explants in the presence and absence of extracellular Na^+ was 2.175 ± 0.478 and 0.239 ± 0.228 nmol/g cell wet weight/2 min respectively ($n = 3$). Therefore, it is evident that bromocryptine treatment reduced the Na^+-dependent moiety of D-aspartate uptake by 81%.

4. Discussion

The experiments described in this paper were designed to examine the regulation of Na^+-dependent anionic amino acid transport by the rat mammary gland. For convenience we used explants as a model to study D-aspartate transport by the mammary gland. Although mammary explants are a mixed cell population, the vast majority are secretory epithelial cells. Moreover, the acinar structure of the gland ensures that the majority of the surface area exposed to the incubation medium will be basolateral membranes of the secretory cells. Therefore, we believe that the changes in D-aspartate transport observed in this study primarily reflect changes in transport across the blood-facing aspect of the mammary epithelium [2].

The results show that Na^+-dependent anionic amino acid transport by rat mammary tissue changes during the lactation cycle in a manner which suggests that the transport of anionic amino acids is regulated to meet the demands of milk protein synthesis. This notion is based on the observation that the pattern of activity of (Na^+-anionic amino acid) cotransport appears to be similar to that of milk yield [18]. Thus, there was a marked increase in D-aspartate transport following parturition which increased further towards peak lactation and declined thereafter in late lactation. The transport of cycloleucine by rat mammary acini exhibits a similar pattern during the lactation cycle [19] suggesting that system L is regulated in a similar fashion. In contrast to the observed changes in D-aspartate transport, we found that the Na^+-dependent uptake of taurine by rat mammary tissue declined between early and peak lactation: the reduction in taurine transport by mammary tissue matches the fall in milk taurine levels [20]. Given that Na^+-dependent D-aspartate transport increases whereas Na^+-dependent taurine uptake decreases between early and peak lactation it appears that the change to the former cannot be attributed to a change in the *trans*-membrane electrochemical Na^+-gradient.

It is evident that removing rats from their litters during peak lactation markedly reduced Na^+-dependent D-aspartate uptake. This is in accordance with the results of Vina et al. [17] who showed that premature weaning significantly reduced the arteriovenous concentration differences for L-glutamate and L-aspartate across the mammary gland. Premature weaning also decreased D-aspartate efflux as a consequence of reversing the *trans*-membrane Na^+-gradient. This suggests that the effect of litter removal on Na^+-dependent D-aspartate transport cannot be attributed to a reduction in the driving force (i.e. the electrochemical Na^+-gradient) for D-aspartate transport. Interestingly, a recent report has shown that the mammary tissue expression of GLAST, a cloned anionic amino acid carrier [21], is significantly decreased by litter removal [22]. However, as yet, there is no information on the precise locus of GLAST in the rat mammary gland, therefore, its contribution to anionic amino acid transport across the basolateral aspect of the mammary epithelium remains unknown.

The effect of premature weaning on D-aspartate uptake may be due to milk accumulation and/or a change in the hormonal environment (e.g. prolactin). The results from the experiments in which the effect of unilateral weaning was examined suggest that milk accumulation per se is able to regulate the transport of anionic amino acids by the rat mammary gland. This is based on the reasonable assumption that the weaned gland has the same hormonal environment as the suckled gland. The present results are in accordance with those of an earlier study which demonstrated that unilateral weaning and hence milk accumulation was able to decrease the arteriovenous concentration differences for both L-glutamate and L-aspartate across the rat mammary gland [17]. It appears that the uptake of anionic amino acids via system X_{AG}^- may be regulated in a fashion which matches supply with demand. Similarly, the uptake

of α-aminoisobutyric acid by rat mammary tissue via systems A and L is also regulated by milk stasis [23]. However, the reduction in amino acid transport following milk accumulation may not be entirely independent from hormonal action given that it has been demonstrated that unilateral weaning leads to a reduction in the number of prolactin receptors in the weaned gland compared to the suckled gland [24]. It should be noted that D-aspartate uptake by mammary explants isolated from the suckled gland from animals which had been unilaterally weaned was significantly lower than D-aspartate uptake by mammary tissue taken from rats during peak lactation. As yet we have no clear explanation for this result except for the possibility that there may be signalling between the weaned and suckled glands of the unilaterally weaned rats.

Treating rats during peak lactation with bromocryptine reduced Na^+-dependent D-aspartate uptake by mammary tissue explants which is consistent with the notion that prolactin regulates the transport of anionic amino acids by the mammary gland. The present results are in agreement with the findings of Vina et al. [25] who demonstrated that bromocryptine treatment reduces the arteriovenous concentration difference of L-glutamate across the rat mammary gland although an earlier report from the same laboratory failed to find an effect of bromocryptine [26]. Previous studies have shown that the transport of other amino acids by mammary tissue is regulated by prolactin. Thus, the transport of neutral amino acids via systems L and A in rat mammary tissue is regulated by prolactin [2,19]. In addition, the transport of α-aminoisobutyric acid by mouse mammary tissue in culture is stimulated by prolactin [27]. However, it is not yet known if prolactin has a direct effect on these transport systems or whether the effect is secondary to other metabolic events. Therefore, caution must be exercised in interpreting the effect of prolactin on membrane transport systems in such a highly regulated epithelium.

Acknowledgements

The authors are grateful to Miss C. Burns for expert technical assistance. This work was funded by the Scottish Office Agriculture, Environment and Fisheries Department.

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