Involvement of γ -glutamyltransferase in amino-acid uptake by the lactating mammary gland of the rat

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1. Arteriovenous differences of amino acids across the lactating mammary gland have been measured in normal rats and in rats injected with serine–borate (an inhibitor of γ -glutamyltransferase). 2. Comparison of the arteriovenous differences show that γ -glutamyltransferase is involved in amino-acid uptake by the gland. 3. Reduced-glutathione content of isolated acini incubated with high concentrations of amino acids was lower than that of the controls. 4. High concentrations of amino acids had no effect on reduced-glutathione content of isolated acini when serine–borate was added to the incubation medium. 5. The findings provide evidence for the functioning of the γ -glutamyl cycle in the lactating mammary gland *in vivo*.

The role of γ -glutamyltransferase in amino-acid translocation (as part of the γ -glutamyl cycle) was proposed by Meister (1973). However, direct evidence for the involvement of γ -glutamyltransferase in amino-acid uptake by cells was lacking (Meister, 1978). In the present paper we show that amino-acid uptake by the lactating mammary gland is decreased when γ -glutamyltransferase is inhibited. We also show that GSH content of isolated acini incubated with high concentrations of amino acids is decreased when compared with that of the controls. The findings support the concept of the functioning *in vivo* of the γ -glutamyl cycle in the lactating mammary gland.

Materials and methods

Animals

Wistar rats were fed *ad libitum* on a standard diet for rats and mice from Prada, Vara de Quart, Valencia, Spain. All experiments were started between 10:00 h and 11:00 h. In all cases the rats were in day 16 of lactation and had seven to ten pups. Rats were anaesthetized with Nembutal (50 mg/kg body wt.). The pups were allowed to continue to suckle before the operation.

Preparation of isolated acini

Acini were isolated from mammary glands of rats in day 16 of lactation by the method of Katz *et al.*

Abbreviation used: GSH, reduced glutathione.

(1974) as modified by Robinson & Williamson (1977).

Measurement of GSH

GSH was measured by the glyoxalase method of Racker (1951) under the conditions described previously (Viña *et al.*, 1978).

Analysis of amino acids

For amino-acid analysis, venous blood was collected from the pudic epigastric veins into a heparinized syringe and then arterial blood was collected from the left ventricle into another heparinized syringe. After centrifugation (10 min, 3500 rev./min), plasma was collected and protein was precipitated by mixing 1 ml of plasma and 4 ml of 3.75% (w/v) sulphosalicylic acid in 0.3 M-lithium citrate buffer (pH 2.8). One ml of the supernatant was collected and injected into an LKB 3201 amino-acid analyser for determination of amino acids. Results are expressed as means $\pm$ S.D. for the numbers of observations in parentheses.

Results

Amino-acid uptake by the lactating mammary gland

The concentration of amino acids in arterial plasma, in plasma of blood leaving the mammary gland via the pudic epigastric vein, the arteriovenous differences and the arteriovenous-difference/arterial-concentration-of-amino-acid ratios are shown in Table 1.

To test whether γ -glutamyltransferase is involved in amino-acid uptake by the mammary gland, we

Table 1. *Uptake of amino acids by mammary gland of rats in the 16th day of lactation*

For details see the text. Values are means $\pm$ s.d. for six experiments. Arteriovenous differences that are significant are indicated by: * $P < 0.01$; † $P < 0.05$.

Amino acid	Arterial concentration (μM)	Venous concentration (μM)	Arteriovenous differences (nmol/ml)	Arteriovenous-difference/arterial-concentration-of-amino-acid ratio
Aspartic acid	17 $\pm$ 5	14 $\pm$ 6	3	0.18
Threonine	306 $\pm$ 83	214 $\pm$ 78	92†	0.30
Serine	225 $\pm$ 40	159 $\pm$ 59	66†	0.29
Asparagine	33 $\pm$ 8	22 $\pm$ 10	11†	0.33
Glutamic acid	42 $\pm$ 9	30 $\pm$ 13	12†	0.29
Glutamine	645 $\pm$ 85	284 $\pm$ 51	361*	0.56
Proline	223 $\pm$ 34	155 $\pm$ 58	66†	0.30
Glycine	234 $\pm$ 22	142 $\pm$ 47	92*	0.39
Alanine	423 $\pm$ 80	295 $\pm$ 89	128†	0.30
Valine	257 $\pm$ 63	145 $\pm$ 56	112*	0.44
Cystine	128 $\pm$ 30	68 $\pm$ 26	60*	0.47
Methionine	66 $\pm$ 4	44 $\pm$ 17	22*	0.33
Isoleucine	95 $\pm$ 21	45 $\pm$ 15	50*	0.53
Leucine	188 $\pm$ 39	94 $\pm$ 34	94*	0.50
Tyrosine	88 $\pm$ 33	50 $\pm$ 28	34†	0.43
Phenylalanine	57 $\pm$ 10	28 $\pm$ 9	29*	0.51
Histidine	267 $\pm$ 95	178 $\pm$ 124	89	0.33

Table 2. *Uptake of amino acids by mammary gland of rats in day 16 of lactation, injected with serine-borate (20 mmol/kg body wt.)*

Rats were injected intraperitoneally with serine-borate 1 h before the operation. For details see the text. Results are means $\pm$ s.d. for six experiments. Arteriovenous differences that are significant are indicated by: * $P < 0.01$; † $P < 0.05$.

Amino acid	Arterial concentration (μM)	Venous concentration (μM)	Arteriovenous differences (nmol/ml)	Arteriovenous-difference/arterial-concentration-of-amino-acid ratio
Aspartic acid	22 $\pm$ 9	18 $\pm$ 8	4	0.18
Threonine	167 $\pm$ 60	144 $\pm$ 49	23	0.14
Serine	—	—	—	—
Asparagine	35 $\pm$ 2	25 $\pm$ 5	10*	0.29
Glutamic acid	48 $\pm$ 20	40 $\pm$ 16	8	0.17
Glutamine	214 $\pm$ 34	193 $\pm$ 43	21	0.10
Proline	146 $\pm$ 17	123 $\pm$ 19	23†	0.16
Glycine	91 $\pm$ 31	66 $\pm$ 31	25	0.28
Alanine	294 $\pm$ 36	245 $\pm$ 37	49†	0.17
Valine	212 $\pm$ 14	140 $\pm$ 48	72*	0.34
Cystine	33 $\pm$ 5	28 $\pm$ 7	5	0.15
Methionine	40 $\pm$ 5	37 $\pm$ 6	3	0.07
Isoleucine	77 $\pm$ 22	37 $\pm$ 6	40*	0.52
Leucine	174 $\pm$ 75	105 $\pm$ 71	69	0.40
Tyrosine	112 $\pm$ 66	98 $\pm$ 70	14	0.13
Phenylalanine	38 $\pm$ 7	33 $\pm$ 7	5	0.13
Histidine	308 $\pm$ 62	181 $\pm$ 49	127*	0.41

studied the arteriovenous differences of amino acids in rats injected intraperitoneally with serine-borate (20 mmol/kg body wt.), an inhibitor of γ -glutamyl-transferase (Revel & Ball, 1959; Tate & Meister, 1978). The concentration of amino acids in arterial plasma, in plasma of blood obtained from the pudic epigastric vein, the arteriovenous differences and the arteriovenous-difference/arterial-concentration-of-

amino-acid ratios are shown in Table 2. It is important to observe that in these rats the uptake of amino acids, that are good substrates for γ -glutamyl-transferase, such as glutamine, methionine, cystine and alanine, was greatly diminished (i.e. glutamine uptake fell from 361 nmol/ml of plasma in the controls to 21 nmol/ml of plasma in the rats treated with serine-borate). However, for amino acids that

Table 3. *Effect of high concentration of amino acids on glutathione content of isolated acini of lactating mammary gland*
 Acini were incubated for 60 min. For details see the text. Initial GSH concentration was 11.83 ± 1.06 (17) $\mu\text{mol/g}$ dry wt. The number of observations refer to different acini preparations. The significance test refers to the comparison of GSH content with and without added amino acids: * $P < 0.01$; †Non-significant difference.

Amino-acid added (mM)	GSH content	
	($\mu\text{mol/g}$ dry wt.)	(% of control)
None (control)	10.32 ± 0.72 (9)	100
Gln (20)	7.82 ± 0.57 (7)*	75
Gln (20) + Ser-borate (20)	9.87 ± 0.75 (4)†	95
Ala (20)	7.56 ± 0.55 (6)*	73
Ala (20) + Ser-borate (20)	9.12 ± 0.67 (4)*	88
Gln (20) + Ala (20)	7.44 ± 0.76 (6)*	72
Gln (20) + Ala (20) + Ser-borate (20)	10.05 ± 0.26 (4)†	97
Met (20)	7.23 ± 0.83 (6)*	70
Met (20) + Ser-borate (20)	9.73 ± 0.74 (4)†	94

are poor substrates for the γ -glutamyltransferase the arteriovenous differences and the arteriovenous-difference/arterial-concentration-of-amino-acid ratios in rats treated with serine-borate were similar to those of the control.

When rats were injected with serine (20 mmol/kg body wt.) or borate (20 mmol/kg body wt.) alone, the arteriovenous differences of amino acids were similar to those of the controls (results not shown). This is in agreement with previous results (Revel & Ball, 1959; Tate & Meister, 1978), which show that γ -glutamyltransferase is inhibited by serine-borate and not by serine or borate independently.

We observed that the arterial concentration of amino-acid substrates for γ -glutamyltransferase was lower in rats treated with serine-borate than in controls. This may be a result of diminished intestinal absorption and increased renal excretion of amino acids owing to lack of activity of γ -glutamyltransferase in intestinal cells (Cornell & Meister, 1976) and in kidney tubular cells respectively (Ormstad *et al.*, 1980).

Effect of amino acids on GSH content of isolated acini from lactating mammary gland

Acini isolated from mammary gland had a GSH content of 11.83 ± 1.06 (17) $\mu\text{mol/g}$ dry wt. After 60 min incubation their GSH content fell to 10.32 ± 0.72 $\mu\text{mol/g}$ dry wt. When acini were incubated (60 min) with high concentrations of the amino acids that are the best substrates for γ -glutamyltransferase (i.e. glutamine, alanine or methionine), their GSH content fell to values lower than those of the controls (Table 3). However, when acini were incubated with high concentrations of amino acids in the presence of serine-borate (20 mM) their GSH content was similar to that of the controls (Table 3), thus showing that the GSH depletion was dependent on the activity of γ -glutamyltransferase. When acini were incubated with high concentrations of amino acid in the presence of borate alone their

GSH content was similar to that of acini incubated with amino acid alone. Addition of serine-borate to control incubations of isolated acini had no effect on their GSH content.

Discussion

The γ -glutamyl cycle has been proposed to function in amino-acid translocation (Meister, 1973). The cycle consists of a series of reactions, involving GSH and γ -glutamyltransferase and it has been shown that the enzymes of the cycle are present in several tissues (Meister, 1978). However, a major problem is that a key enzyme, γ -glutamyltransferase, must react with intracellular glutathione and extracellular amino acids (Hahn *et al.*, 1978). Thus the wide distribution of the enzymes is not sufficient to assess the functioning of the cycle *in vivo*, and direct evidence of the role of GSH and of that of γ -glutamyltransferase in amino-acid translocation was needed.

To show the involvement of GSH in amino-acid uptake by kidney, Griffith *et al.* (1978) studied in mice the effect of the intraperitoneal injection of high doses of amino acids on GSH content of kidney and observed a depletion of GSH subsequent to amino-acid overloading. However, when mice were injected with serine-borate (an inhibitor of γ -glutamyltransferase) together with a large dose of amino acid, kidney GSH content was similar to that of the controls. We have used a similar approach in our studies on the effect of high concentrations of amino acids on GSH content of acini isolated from lactating mammary gland, a tissue with relatively high γ -glutamyltransferase activity (Puente *et al.*, 1979; Pocius *et al.*, 1980).

The steady-state concentration of GSH reflects the balance between synthesis of the tripeptide (catalysed by γ -glutamylcysteine synthetase and GSH synthetase) and its degradation (catalysed by γ -glutamyltransferase). Thus when acini are in-

cubated with small concentrations of amino acids no changes in GSH content occur because the amount of GSH used by γ -glutamyltransferase is rapidly regenerated by synthesis, but when high concentrations of amino acids are used, transpeptidation is increased and GSH content is diminished. However, when acini are incubated with high concentrations of amino acids in the presence of serine–borate, GSH content does not change, thus showing that the depletion of GSH induced by high concentrations of amino acids is due to increased activity of γ -glutamyltransferase. Osuji (1979) showed that GSH turnover in yeast is increased when amino acids are added to the nutrient medium.

However, to show the functioning of the cycle *in vivo* a direct demonstration of the involvement of γ -glutamyltransferase in amino-acid transport into cells was needed (Meister, 1978). The comparison of the arteriovenous differences and of the arteriovenous-difference/arterial-concentration-of-amino-acid ratios in non-treated rats and in rats injected with serine–borate demonstrate that γ -glutamyltransferase is involved in amino-acid translocation in the lactating mammary gland. While this work was in progress, Ormstad *et al.* (1980), studying the characteristics of GSH biosynthesis in rat kidney cells, observed that the uptake by isolated cells of some amino-acid precursors for GSH biosynthesis (glutamate and methionine) was diminished when the cells were incubated with serine–borate.

The lower concentration of some amino acids in arterial blood of rats treated with serine–borate, compared with the arterial concentration of amino acids in the controls, may be due to lack of intestinal absorption owing to the inhibition of intestinal γ -glutamyltransferase.

It is evident that other mechanisms must exist for the translocation of amino acids, mainly for those that are not substrates for γ -glutamyltransferase.

The question of how γ -glutamyltransferase reacts with intracellular GSH and extracellular amino acids is still open, but we have provided evidence that γ -glutamyltransferase is indeed involved in amino-acid uptake. The experiments reported in the present paper strongly support the hypothesis that the γ -glutamyl cycle is functioning in the lactating mammary gland *in vivo*.

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