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Effect of specific inhibition of gamma-glutamyl transpeptidase on amino acid uptake by mammary gland of the lactating rat

José Viña, Inmaculada R. Puertes, Juan B. Montoro and Juan R. Viña

Departamento de Bioquimica y Fisiologia, Facultad de Medicina, Avenida Blasco Ibañez, 17, Valencia-10, Spain

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We showed [Biochem. J. (1981) 194, 99–102] that inhibition of γ-glutamyl transpeptidase in vivo with serine-borate decreases amino acid uptake by mammary gland. However, doubts arose about the validity of this inhibitor in metabolic studies because it must be used in very large amounts. New inhibitors have been isolated, like anthglutin and acivicin, which are effective at low concentrations in vivo. Here, we show that treatment of lactating rats with these substances decreases the transpeptidase activity and the amino acid uptake by the gland. These results support the hypothesis that the γ-glutamyl cycle functions as an amino acid transport system in mammary gland.

Gamma-glutamyl transpeptidase (EC 2.3.2.2) *Amino acid transport* *Glutathione* *Gamma-glutamyl cycle* *Mammary gland (rat)* *Lactation*

1. INTRODUCTION

The concept of the gamma-glutamyl cycle [1] has attracted attention because it postulates a mechanism for amino acid transport which consists of a series of reactions that form a metabolic cycle. However, this concept has been seriously challenged [2]. Indeed, a key enzyme, gamma-glutamyl transpeptidase, whose active centre faces the external part of the cell membrane [3], must react with extracellular amino acids and intracellular glutathione. Thus, direct proof of the role of the gamma-glutamyl cycle as an amino acid transport system was required [4].

We showed [5] that treatment of lactating rats with serine-borate, an inhibitor of gamma-glutamyl transpeptidase [6], decreases amino acid uptake by the gland. However, very large amounts of serine-borate (20 mmol/kg body wt) had to be used. This presents two problems:

(i) That such large amounts might be cytotoxic and thus the decreased amino acid uptake might not represent a specific inhibition of gamma-glutamyl transpeptidase in vivo;

(ii) That unknown amounts of free serine would be present in the mixture of serine and borate.

Recently new substances have been isolated that are inhibitors of gamma-glutamyl transpeptidase [7,8] and that overcome these shortcomings.

Here we show that these substances are effective as inhibitors of gamma-glutamyl transpeptidase in mammary gland in vivo. Treatment of lactating rats with these specific inhibitors of gamma-glutamyl transpeptidase results in a decrease in uptake by the gland of the amino acids that are good substrates of gamma-glutamyl transpeptidase, while those which are poor substrates of the enzyme are not affected, thus providing strong support for the hypothesis that the gamma-glutamyl cycle functions as an amino acid transport system in mammary gland in vivo.

2. MATERIALS AND METHODS

Lactating Wistar rats at the peak of lactation (days 10–15 of lactation) were used. They always had access to food and water.

Arteriovenous differences of amino acids were

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obtained measuring the concentration of amino acids in whole blood from the pudic-epigastric vein and from the aorta, as in [5]. Acini from mammary glands were isolated by the method in [9], under the conditions in [10]. Lipid synthesis was measured as in [11]. Lactate and pyruvate were measured by standard enzymatic methods [12]. Gamma-glutamyl transpeptidase was measured by following the hydrolysis of gamma-glutamyl-*p*-nitro-anilide as in [13].

[1-^{14}C]Glucose was from Amersham International (Bucks). Acivicin (AT-125) was obtained from Dr Hanka, The Upjohn Company (Kalamazoo MI) and anthglutin from Dr Minato, Sankyo Co. (Tokyo).

We used 25 mg anthglutin/kg body wt or 10 mg acivicin/kg body wt, injected i.p., 1 h before measuring the arteriovenous differences of amino acids.

3. RESULTS AND DISCUSSION

3.1. *Effect of acivicin and anthglutin on gamma-glutamyl transpeptidase activity in mammary gland*

The gamma-glutamyl transpeptidase activity in mammary gland at the peak of lactation is 10.9 ± 1.7 (n = 8) μmol *p*-nitroanilide.min^{-1}.g fresh wt^{-1}. When rats were injected with 10 mg/kg body wt acivicin, as in [14], the gamma-glutamyl transpeptidase activity fell to 5.7 ± 2.5 (n = 5) μmol.min^{-1}.g fresh wt^{-1}; i.e., 52% of the controls, in agreement with previous findings in other organs which showed that acivicin is an irreversible inhibitor of gamma-glutamyl transpeptidase. However, when rats were injected with 25 mg/kg body wt of anthglutin and gamma-glutamyl transpeptidase was determined in mammary gland homogenates, the value found was 8.8 ± 2.0 (n = 4); i.e., non-statistically different from controls. This may be explained by the facts that the inhibition of gamma-glutamyl transpeptidase by anthglutin is reversible [7] and that the concentration of anthglutin in the assay system containing a small sample of a mammary gland homogenate is very low. However, when 0.25 mM anthglutin, i.e., a concentration similar to that found in the blood of a 200 g rat injected with 5 mg of anthglutin, was added to the assay system, the gamma-glutamyl transpeptidase activity of mammary gland homogenates fell to 3.6 ± 0.2 (n = 4) μmol.min^{-1}.g fresh wt^{-1}.

Thus, both acivicin and anthglutin proved to be effective inhibitors of gamma-glutamyl transpeptidase in mammary gland, as previously observed with other tissues.

3.2. *Effect of serine-borate on glucose metabolism in mammary gland*

In [5] we used large amounts of the complex serine-borate (20 mmol/kg body wt) to inhibit gamma-glutamyl transpeptidase in rat mammary gland and found that it inhibited amino acid uptake by the gland. However, doubts arose about the validity of this inhibitor in metabolic studies because large concentrations had to be used. Indeed non-specific damage to acinar cells could also result in a decrease in amino acid uptake. We have thus tested the effect of 20 mM serine-borate on glucose metabolism by isolated acini from mammary gland and found that when they were incubated with 5 mM [1-^{14}C]glucose, the formation of lactate, pyruvate and CO_2 and the incorporation of carbon units to lipids by the acini were not affected by incubation with 20 mM serine-borate (table 1), thus showing that, at least in vitro, such large amounts of the inhibitor do not exert toxic effects on glucose and lipid metabolism of acinar cells.

3.3. *Effect of inhibition of gamma-glutamyl transpeptidase on amino acid uptake by mammary gland*

Table 2 shows that inhibition of gamma-glutamyl transpeptidase in mammary gland by treatment with acivicin or anthglutin results in a decrease in arteriovenous differences of amino acids, specially of those that are substrates of gamma-glutamyl transpeptidase while those that are not substrates of the enzyme, like the branched chain, the aromatic and the basic amino acids, are not affected. In rats treated with anthglutin, however, the uptake by the gland of proline, lysine and arginine, which are poor substrates of gamma-glutamyl transpeptidase, was decreased. We do not have an explanation for these 3 exceptions, probably anthglutin might exert other effects apart from inhibiting gamma-glutamyl transpeptidase. However, the fact that all the amino acids that are good substrates of the transpeptidase are affected

Table 1

Effect of serine-borate on [1-^{14}C]glucose metabolism in isolated acini from mammary glands of lactating rats

Additions	$^{14}CO_2$ formed	[^{14}C]Lipids formed	Lactate formed	Pyruvate formed
[1-^{14}C]Glucose	0.46 ± 0.09	0.27 ± 0.04	0.26 ± 0.11	0.07 ± 0.02
[1-^{14}C]Glucose + serine-borate	0.44 ± 0.03	0.26 ± 0.02	0.33 ± 0.11	0.08 ± 0.02

For details see section 2. The incubation medium contained glucose (5 mM) and serine-borate (20 mM). The results are mean values ± SD, expressed as μmol.min^{-1}.100 mg dry wt^{-1}, for 4 expt

by anthglutin, that the majority of those that are not substrates are not affected and the fact that with acivicin, the other inhibitor used, only the amino acids that are good substrates of the enzyme are affected while those that are poor substrates are not, provides strong support for the theory that the gamma-glutamyl cycle indeed functions as an amino acid transport system in mammary gland.

Recently [15], it has been emphasized that selective modifications of glutathione metabolism may

Table 2

Effects of anthglutin and acivicin on blood arteriovenous differences of amino acids across the mammary gland of lactating rats

Amino acid	Controls (n = 6)		Anthglutin-treated (n = 4)		Acivicin-treated (n = 6)	
	Arterial level	Arteriovenous differences	Arterial level	Arteriovenous differences	Arterial level	Arteriovenous differences
L-Aspartic acid	36 ± 5	10 ± 2	37 ± 10	4 ± 1[b]	41 ± 9	4 ± 1[b]
L-Threonine	432 ± 98	108 ± 14	241 ± 103	23 ± 20[b]	397 ± 53	21 ± 9[b]
L-Serine	340 ± 81	82 ± 27	216 ± 69	19 ± 12[a]	321 ± 23	27 ± 10[a]
L-Asparagine	60 ± 11	18 ± 6	62 ± 18	12 ± 4	66 ± 6	8 ± 3[a]
L-Glutamic acid	213 ± 16	29 ± 5	177 ± 62	11 ± 7[a]	194 ± 20	11 ± 4[b]
L-Glutamine	589 ± 35	138 ± 30	563 ± 216	52 ± 20[b]	629 ± 96	54 ± 19[b]
L-Proline	266 ± 49	47 ± 14	203 ± 52	19 ± 2[a]	237 ± 30	29 ± 9
Glycine	301 ± 71	58 ± 15	216 ± 62	22 ± 9[a]	281 ± 36	24 ± 7[b]
L-Alanine	546 ± 102	131 ± 15	569 ± 236	51 ± 41[a]	633 ± 114	34 ± 13[b]
L-Valine	170 ± 37	69 ± 24	191 ± 68	35 ± 14	188 ± 38	62 ± 9
L-Cystine	137 ± 15	41 ± 8	116 ± 29	23 ± 7	112 ± 10	17 ± 3[b]
L-Methionine	115 ± 11	40 ± 8	85 ± 19	19 ± 3[a]	90 ± 13	15 ± 4[b]
L-Isoleucine	113 ± 12	45 ± 10	75 ± 27	24 ± 8	80 ± 11	29 ± 8
L-Leucine	191 ± 20	71 ± 20	164 ± 54	47 ± 9	178 ± 26	73 ± 11
L-Tyrosine	123 ± 21	36 ± 11	131 ± 26	14 ± 7	136 ± 26	25 ± 6
L-Phenylalanine	41 ± 9	15 ± 5	46 ± 10	13 ± 6	51 ± 5	16 ± 6
L-Lysine	139 ± 11	29 ± 5	125 ± 4	15 ± 2[b]	124 ± 4	16 ± 9
L-Histidine	203 ± 11	35 ± 9	167 ± 12	18 ± 7	184 ± 12	24 ± 11
L-Arginine	63 ± 2	20 ± 4	64 ± 10	10 ± 3[a]	76 ± 10	20 ± 6

For details see text. Results are mean ± SD, expressed as nmol/ml, with the numbers of experiments in parentheses. Arteriovenous differences that are significantly different from the control are shown: [a] $p < 0.005$; [b] $p < 0.001$

throw light on several functions of glutathione. Here we show that selective inhibition of gamma-glutamyl transpeptidase decreases amino acid uptake by the gland in vivo, independently of the inhibitor used. We have shown [16,17] that changes in gamma-glutamyl transpeptidase activity induced by prolactin or oestrogens are followed by parallel changes in amino acid uptake by the gland. This, together with the facts reported here, suggests that the regulation of gamma-glutamyl cycle as an amino acid transport mechanism may be exerted through changes in gamma-glutamyl transpeptidase activity.

The fact that the inhibition of gamma-glutamyl transpeptidase affects the uptake of some amino acids, but not all of them, emphasizes the idea that the gamma-glutamyl cycle may be one of the several amino acid transport systems that occur in the cell, and that its function does not exclude the existence of other amino acid transport mechanisms.

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