ROLE OF PROLACTIN IN AMINO ACID UPTAKE BY THE LACTATING MAMMARY GLAND OF THE RAT

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1. Introduction

The mammary gland is under hormonal control during lactation. We reported that γ-glutamyl transferase and glutathione are involved in amino acid uptake by the lactating mammary gland [1].

In [2,3] it was shown that γ-glutamyl transferase activity in the mammary gland is subject to hormonal control. Thus, we investigated the hormonal regulation of amino acid uptake by the mammary gland of the rat and the evolution of arteriovenous differences of amino acids across the mammary gland during lactation.

Here we report that changes in arteriovenous differences of amino acids across the mammary gland parallel those of γ-glutamyl transferase activity and that prolactin plays an important role in the regulation of amino acid uptake by the mammary gland.

2. Materials and methods

2.1. *Animals*

Wistar rats (200–250 g) were fed ad libitum on a standard diet for rats and mice from Prasa, Vara de Quart, Valencia. All experiments were started between 10:00–11:00 h. In all cases the rats had 7–10 pups. Prolactin deficiency was induced by injection (i.p.) of bromocryptine (10 mg/kg body wt; solution in 10% (v/v) ethanol containing 1% (w/v) tartaric acid) 24 h before the experiments [4,5]. Where indicated, rats were treated with bromocryptine as above and, 2 and 9 h after bromocryptine injection, with bovine prolactin (injection i.p. of 2 mg in 0.2 ml 154 mM NaCl, pH 10). In these cases the collections of blood samples were made 24 h after the first prolactin injection [6]. Rats were anaesthetized with Nembutal (50 mg/kg body wt). The pups were allowed to continue to suckle before collecting blood samples [7].

2.2. *Analysis of amino acids*

For amino acid analysis, venous blood was collected from the pudic epigastric veins into a heparinized syringe and arterial blood was collected from the left ventricle into another heparinized syringe. After centrifugation (10 min, 3500 rev./min) plasma was collected and amino acids were determined as in [1]. Results are expressed as means ± SD for no. obs. in parentheses.

2.3. *Chemicals*

Bromocryptine was a gift of Dr F. Pallardó (Valencia). Bovine prolactin was from Sigma Chemical Co. (London). All other chemicals were of highest purity available.

3. Results and discussion

3.1. *Evolution of arteriovenous differences of amino acids, across mammary gland during lactation*

Table 1 shows the arteriovenous differences of amino acids through the mammary gland at the day of birth of the pups (day 0 of lactation) and at days 5, 10, 15 and 20 of lactation.

The arteriovenous differences increase from day 0–5 and from day 5–10. They remain high between days 10–15, but fall sharply at day 20 (see table 1). These changes parallel those of the activity of γ-glutamyl transferase in mammary gland during lactation [2,3] and provide additional evidence for the role of γ-glutamyl transferase in amino acid uptake by the

Table 1
Evolution of arteriovenous differences of amino acids across the mammary gland during lactation

Amino acid	Days of lactation				
	0 (6)	5 (4)	10 (4)	15 (6)	20 (4)
Asp	2 ± 2	8 ± 5	6 ± 2	3 ± 2	5 ± 2
Thr	97 ± 6	124 ± 47	205 ± 22	92 ± 20	67 ± 13
Ser	33 ± 8	52 ± 25	143 ± 49	66 ± 27	62 ± 39
Asn	6 ± 3	15 ± 9	14 ± 6	15 ± 9	6 ± 1
Glu	11 ± 2	18 ± 8	18 ± 2	12 ± 7	12 ± 6
Gln	170 ± 33	316 ± 49	333 ± 73	361 ± 97	41 ± 17
Pro	61 ± 4	89 ± 42	89 ± 27	66 ± 34	39 ± 3
Gly	50 ± 18	87 ± 22	178 ± 58	92 ± 3	14 ± 9
Ala	46 ± 27	90 ± 24	119 ± 18	160 ± 45	32 ± 4
Val	23 ± 7	74 ± 44	–	112 ± 9	40 ± 10
Cys	21 ± 9	35 ± 13	48 ± 8	60 ± 24	22 ± 7
Met	12 ± 3	19 ± 8	40 ± 8	22 ± 18	10 ± 2
Ile	19 ± 8	38 ± 12	74 ± 6	50 ± 10	15 ± 7
Leu	47 ± 9	107 ± 32	103 ± 7	94 ± 10	68 ± 16
Tyr	17 ± 7	25 ± 12	30 ± 8	38 ± 7	26 ± 10
Phe	12 ± 5	21 ± 8	14 ± 4	29 ± 3	11 ± 3
Lys	27 ± 7	53 ± 29	–	195 ± 57	49 ± 39
His	24 ± 10	50 ± 16	88 ± 16	89 ± 34	26 ± 5
Arg	12 ± 7	20 ± 4	16 ± 5	15 ± 2	12 ± 5

For details see the text. Arteriovenous differences are expressed as nmol/ml. Results are means ± SD for no. expt. in parentheses

lactating mammary gland [1]. Thus, arteriovenous differences of amino acids were maximal at the peak of lactation; i.e., from days 10–15 after birth. It should be noted that arteriovenous differences of plasma amino acids across the mammary gland are maximal in the case of glutamine, threonine, serine, glycine, alanine and leucine.

Amino acid uptake depends on blood flow through the organ as well as on arteriovenous differences. Since at the peak of lactation both the blood flow [8] and the arteriovenous differences of amino acids (table 1) are maximal, we conclude that the uptake of amino acids by the gland is maximal between days 10–15 after birth, when specific direction of blood metabolites to the mammary gland is maximal [9,10].

3.2. *Role of prolactin on amino acid uptake by the lactating mammary gland*

A possible candidate for the regulation of amino acid uptake by the mammary gland was prolactin. To test this, we compared the arteriovenous differences through the mammary gland under 3 different situations:

(i) Normal lactating rats at the peak of lactation (days 10–15) when amino acid uptake is maximal (see table 1);
(ii) Rats at the peak of lactation injected with bromocryptine;
(iii) Rats at the peak of lactation injected with bromocryptine plus prolactin.

Table 2 shows that treatment with bromocryptine induced a marked decrease in arteriovenous differences of amino acids and that in the case of rats treated with bromocryptine plus prolactin the arteriovenous differences of amino acids are similar to those of the controls for all amino acids except threonine, isoleucine and leucine. The reason for these 3 exceptions remains to be established.

In [3] γ-glutamyl transferase activity in mammary gland was induced by prolactin. In [1] γ-glutamyl transferase was reported to be involved in amino acid uptake by the mammary gland. Here, we report that prolactin plays major role in the regulation of the uptake of amino acids by the gland by affecting the arteriovenous differences of amino acids. The role of changes of blood flow through the mammary gland

Table 2
Effect of bromocryptine and prolactin on amino acid uptake by mammary gland of rats between day 10–15 of lactation

Amino acid	Controls (11)		Bromocryptine-treated (6)		Bromocryptine + prolactin-treated (4)	
	Arterial level (μM)	Arteriovenous differences (nmol/nl)	Arterial level (μM)	Arteriovenous differences (nmol/ml)	Arterial level (μM)	Arteriovenous differences (nmol/ml)
Asp	21 ± 6	6 ± 3	26 ± 6	4 ± 1	31 ± 8	7 ± 1
Thr	456 ± 191	152 ± 57	392 ± 50	59 ± 44[a]	391 ± 43	45 ± 8[a]
Ser	315 ± 87	88 ± 41	356 ± 25	47 ± 18[a]	342 ± 65	154 ± 30
Asn	40 ± 10	14 ± 5	47 ± 6	8 ± 2[a]	44 ± 4	17 ± 6
Glu	51 ± 10	17 ± 9	62 ± 7	12 ± 5	66 ± 10	22 ± 4
Gln	708 ± 98	336 ± 71	640 ± 34	43 ± 20[b]	571 ± 21	209 ± 40[a]
Pro	265 ± 43	119 ± 36	258 ± 37	39 ± 21[b]	293 ± 33	111 ± 42
Gly	314 ± 142	151 ± 52	242 ± 34	28 ± 12[b]	279 ± 49	72 ± 18[a]
Ala	564 ± 151	144 ± 32	629 ± 37	39 ± 23[b]	578 ± 98	123 ± 25
Val	219 ± 61	71 ± 42	218 ± 16	42 ± 15	234 ± 60	70 ± 46
Cys	157 ± 48	60 ± 21	152 ± 36	31 ± 17[a]	174 ± 16	59 ± 10
Met	86 ± 17	35 ± 10	94 ± 10	17 ± 8[a]	116 ± 15	35 ± 9
Ile	130 ± 40	76 ± 20	107 ± 15	27 ± 11[b]	108 ± 18	30 ± 10[b]
Leu	223 ± 40	102 ± 11	215 ± 25	74 ± 36[a]	211 ± 55	76 ± 15[a]
Tyr	122 ± 35	33 ± 8	121 ± 22	15 ± 6[b]	132 ± 44	25 ± 7
Phe	55 ± 9	21 ± 7	54 ± 6	12 ± 4[a]	66 ± 33	23 ± 8
Lys	419 ± 229	107 ± 85	156 ± 43	25 ± 9[a]	173 ± 55	54 ± 23
His	229 ± 63	78 ± 29	152 ± 7	26 ± 10[b]	186 ± 37	59 ± 13
Arg	45 ± 21	16 ± 4	46 ± 3	8 ± 3[b]	53 ± 7	17 ± 7

For details see the text. Results are means ± SD for the no. expt. parentheses. Arteriovenous differences that are statistically different from the controls are shown: $^{a}P < 0.05$; $^{b}P < 0.005$

in bromocryptine induced prolactin deficiency remains to be established. However, a decrease in blood flow through the mammary gland of prolactin deficient rats would mean a further decrease in amino acid uptake by the gland.

During lactation, amino acids are required for casein synthesis and it has also been observed that some, i.e., leucine, are good precursors for lipid synthesis [11]. Thus, the role of prolactin in the regulation of amino acid uptake by the mammary gland may be of physiological importance.

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References

[1] Viña, J., Puertes, I. R., Estrela, J. M., Viña, J. R. and Galbis, J. L. (1981) Biochem. J. 194, 99–102.

[2] Puente, J., Varas, M. A., Beckhaus, G. and Sapag-Hagar, M. (1979) FEBS Lett. 99, 215–218.

[3] Pocius, P. A., Baumrucker, C. R., McNamara, J. P. and Bauman, D. E. (1980) Biochem. J. 188, 565–568.

[4] Seki, M., Seki, K., Yoshihara, T., Watonabe, N. Okumura, T., Tajima, C., Huang, S.-Y. and Kuo, L.-C. (1974) Endocrinology 94, 911–914.

[5] Robinson, A. M. and Williamson, D. H. (1977) Biochem. J. 164, 153–159.

[6] Agius, L., Robinson, A. M., Girard, J. R. and Williamson, D. H. (1979) Biochem. J. 180, 689–692.

[7] Amenomori, Y., Chen, C. L. and Meites, J. (1970) Endocrinology 86, 506–510.

[8] Chatwin, A. L., Linzell, J. L. and Setchell, B. P. (1969) J. Endocrinol. 44, 247–254.

[9] Williamson, D. H. (1973) Soc. Exp. Biol. Symp. 27, 283–298.

[10] Williamson, D. H. (1980) FEBS Lett. 117 suppl., K93–K105.

[11] Viña, J. R. and Williamson, D. H. (1981) Biochem. J. 194, 941–947.