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Effect of premature weaning on amino acid uptake by the mammary gland of lactating rats

Juan R. VIÑA, Inmaculada R. PUERTES and José VIÑA
Departamento de Bioquimica y Fisiologia, Facultad de Medicina, Avenida Blasco Ibañez 17, Valencia-10, Spain

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1. Arteriovenous differences of amino acids across the lactating mammary gland were measured in normal rats and rats weaned for 4, 5 and 24h. 2. Uptake of amino acids by mammary glands of rats weaned for 5h or more was significantly lower than that of controls. This was not reversed by injection of prolactin. 3. By using 'unilaterally weaned' rats we showed that milk accumulation plays an important role in amino acid uptake by mammary gland. 4. γ-Glutamyltransferase activity was signfiicantly lower in 'weaned' glands than in 'normal' glands. This provides further support for the hypothesis of the function of the γ-glutamyl cycle in the mammary gland *in vivo*.

At the peak of lactation the mammary gland of the rat takes up considerable amounts of amino acids (Viña *et al.*, 1981*a*). During this period the mammary gland becomes an important site of protein synthesis, and it has been observed that some amino acids taken up by the gland, i.e. L-leucine and L-alanine, are good precursors for lipid synthesis (Viña & Williamson, 1981*a*,*b*).

We reported that γ-glutamyltransferase (γ-glutamyl transpeptidase, EC 2.3.2.2) and glutathione are involved in amino acid translocation into the lactating mammary gland (Viña *et al.*, 1981*a*). We also reported that prolactin plays an important role in the regulation of amino acid uptake by the lactating mammary gland (Viña *et al.*, 1981*b*).

The present paper is concerned with the effects of premature weaning on amino acid uptake by the gland. A novel finding is that milk accumulation plays an important role in amino acid uptake by mammary gland independently of hormonal regulation and of blood flow through the glands.

Material and methods

Rats

Lactating rats of the Wistar strain (200–250g) were fed *ad libitum* on standard diet for rats and mice (Prasa, Vara de Quart, Valencia, Spain). In all cases the rats had between eight and ten pups. Rats were anaesthetized with Nembutal (50mg/kg body wt.).

Rats at the peak of lactation were separated from their young by removing the pups from the mother's cage and are referred to in the text as 'weaned' rats. Three groups were studied at 4, 5 and 24h after separation. Where indicated bovine prolactin (2mg in 0.2ml of 154mM-NaCl, pH10) was injected subcutaneously 2h after the removal of pups. Normal lactating rats at peak of lactation served as controls.

To prevent milk removal by suckling, another group of rats had the teats of one side sealed with adhesive tape for 5h before measurement of the arteriovenous differences of amino acids. These rats were used as a model, having 'normal' and 'weaned' glands in the same animal, and thus the same hormonal environment and blood flow (Hanwell & Linzell, 1973), and are referred to in the text as 'unilaterally weaned rats'.

Measurement of γ-glutamyltransferase activity

γ-Glutamyltransferase activity was measured by following the reaction of γ-glutamyl *p*-nitroanilide and glycylglycine as described by Tate & Meister (1974).

Analysis of amino acids

For measurements of arteriovenous differences of amino acids across the lactating mammary gland, venous blood was collected from the pudic–epigastric vein into a heparinized syringe and arterial blood from the abdominal aorta into another heparinized syringe.

Protein was precipited by mixing 1ml of whole blood and 4ml of 3.75% (w/v) sulphosalicyclic acid

in 0.3 M-lithium citrate buffer (pH 2.8). A sample (1 ml) of the supernatant was collected and injected into an LKB 3201 amino acid analyser for the determination of amino acids.

Results and discussion

Effect of premature weaning on arteriovenous differences of amino acids through the lactating mammary gland

Arteriovenous differences of amino acids were measured across the mammary gland of rats at the peak of lactation and after the removal of pups for 4, 5 and 24 h (Table 1).

The amount of amino acids extracted by the mammary gland of rats weaned for 4 h was not significantly different from that of controls, except for methionine and isoleucine. However, in rats weaned for 5 or 24 h the arteriovenous differences fell to values significantly lower than in controls (Table 1). Amino acid uptake can be calculated from arteriovenous differences and blood flow. It is well established that weaning for 5 h or more decreases blood flow (Hanwell & Linzell, 1973), and we have shown that arteriovenous differences are also diminished. Thus net amino acid uptake by the gland will be greatly impaired by weaning.

The 'signals' that bring about the inhibition of amino acid uptake by the lactating mammary gland between 4 and 5 h after removal of pups could be of the following nature.

(*a*) A decrease in plasma prolactin after removal of pups for 5 h.

(*b*) Lactating rats weaned for 5 h have a marked fall in mammary blood flow (Hanwell & Linsell, 1973). Owing to the decrease in mammary blood flow, the rate of delivery of hormones to the tissue will be decreased and receptor amounts for prolactin and oestrogens will be affected (Moore & Forsyth, 1980).

(*c*) The accumulation of milk in the lactating mammary gland could induce, by an unknown mechanism, the inhibition of amino acid uptake. Indeed, milk accumulation inhibits fatty acid synthesis by mammary gland (Levy, 1964).

Prolactin plays an important role in the regulation of amino acid uptake by the lactating mammary gland (Viña *et al.*, 1981*b*). However, injection of prolactin 2 h after separation of pups from the mother did not affect the fall in the removal of amino acids induced by 5 h of weaning (results not shown). This experiment showed that prolactin deficiency cannot be the only factor that causes the inhibition of amino acid removal induced by 5 h of weaning. Thus an experimental model was needed to study the main factors that were responsible for the inhibition of the amino acid uptake by the lactating mammary gland.

Table 1. *Effect of weaning on arteriovenous differences of amino acids through lactating mammary gland*

For details see the text. Values are means ± S.D., expressed as nmol/ml, with the numbers of experiments in parentheses. Arteriovenous differences that are significantly different from the control are shown: $*P < 0.05$, $**P < 0.005$.

	Arteriovenous difference (nmol/ml)			
Period of weaning (h) ...	Control	4	5	24
Amino acid	(6)	(6)	(5)	(5)
Aspartic acid	10 ± 2	6 ± 2*	2 ± 1**	2 ± 1**
Threonine	108 ± 14	81 ± 18*	37 ± 11**	13 ± 10**
Serine	82 ± 27	72 ± 23	36 ± 38*	−1 ± 18**
Asparagine	18 ± 6	18 ± 3	5 ± 2**	2 ± 2**
Glutamic acid	29 ± 5	29 ± 3	20 ± 10	13 ± 7**
Glutamine	138 ± 30	151 ± 24	67 ± 56*	55 ± 11**
Proline	47 ± 14	59 ± 11	20 ± 12*	19 ± 9**
Glycine	58 ± 15	37 ± 10*	20 ± 19**	2 ± 16**
Alanine	131 ± 15	125 ± 32	14 ± 42**	−7 ± 19**
Valine	69 ± 24	47 ± 17	29 ± 23*	11 ± 13**
Cysteine	41 ± 8	35 ± 9	19 ± 16*	3 ± 7**
Methionine	40 ± 8	24 ± 6**	11 ± 3**	7 ± 6**
Isoleucine	45 ± 10	28 ± 2**	17 ± 11**	10 ± 7**
Leucine	71 ± 20	71 ± 13	30 ± 26*	13 ± 9**
Tyrosine	36 ± 11	32 ± 5	11 ± 3**	14 ± 8**
Phenylalanine	15 ± 5	10 ± 3*	10 ± 10	3 ± 3**
Lysine	29 ± 5	22 ± 4*	10 ± 5**	6 ± 3**
Histidine	35 ± 9	33 ± 5	15 ± 14*	6 ± 6**
Arginine	20 ± 4	14 ± 3*	6 ± 4**	5 ± 2**

Amino acid uptake by 'normal' and 'weaned' glands in the same lactating rat

To find out if milk accumulation can induce a decrease in amino acid uptake by mammary gland, we used 'unilaterally weaned' rats, i.e. normal rats at the peak of lactation, with the teats of one side sealed with adhesive tape for 5h to prevent the removal of milk by suckling. Hanwell & Linzell (1973) used a similar model of 'unilaterally weaned' rats, by sealing the teat ducts of one side with adhesive. Although, at least in theory, this should allow suckling from the 'weaned glands' to continue, there should not be significant differences with our model, in which the glands are sealed with adhesive tape, because we allowed the pups to remain suckling from the normal side and to keep in touch with the sealed glands. No changes in blood flow through the 'weaned glands' are expected, because prolactin concentrations are maintained, and thus there should not be cardiac output changes in the rat and capillary closure does not occur until the milk has accumulated in the gland for 36–48h (Silver, 1956). With this experimental model we have shown that the arteriovenous differences of amino acids in the normal side were similar to those of normal lactating rats (Tables 1 and 2). In contrast, the differences in the side which had the teats sealed were significantly lower than those of normal glands (Table 2). Thus milk accumulation plays an important role in amino acid uptake by mammary gland independently of hormonal regulation and of blood flow through the glands.

Table 2. *Uptake of amino acids by 'unilaterally weaned' rats*
For details see the text. Values are means ± S.D. for six experiments. Arteriovenous differences that are significantly different between intact mammary glands and glands sealed with tape are shown: $*P < 0.05$, $**P < 0.005$.

Amino acid	Arterial concn. (μM)	Arteriovenous differences (nmol/ml) Intact mammary glands	Arteriovenous differences (nmol/ml) Mammary glands sealed with tape
Aspartic acid	39 ± 7	10 ± 4	4 ± 2**
Threonine	376 ± 52	91 ± 22	36 ± 14**
Serine	349 ± 99	90 ± 42	48 ± 36**
Asparagine	64 ± 4	21 ± 4	8 ± 6**
Glutamic acid	215 ± 50	49 ± 20	17 ± 16**
Glutamine	682 ± 67	183 ± 59	46 ± 27**
Proline	255 ± 28	84 ± 41	31 ± 28*
Glycine	262 ± 94	79 ± 29	8 ± 6**
Alanine	594 ± 37	132 ± 23	52 ± 31**
Valine	164 ± 25	62 ± 14	28 ± 6**
Cysteine	136 ± 25	51 ± 14	23 ± 16**
Methionine	97 ± 19	38 ± 9	11 ± 7**
Isoleucine	99 ± 8	38 ± 10	16 ± 9**
Leucine	221 ± 34	87 ± 20	37 ± 19**
Tyrosine	129 ± 41	44 ± 29	19 ± 15*
Phenylalanine	42 ± 11	13 ± 6	7 ± 4*
Lysine	126 ± 17	33 ± 25	14 ± 9*
Histidine	208 ± 14	49 ± 37	17 ± 13*
Arginine	59 ± 3	20 ± 6	7 ± 5**

Effect of premature weaning on γ-glutamyltransferase activity in lactating mammary gland

The hypothesis of the role of γ-glutamyltransferase in amino acid uptake by cells was proposed by Meister (1973). We have shown that γ-glutamyltransferase is indeed involved in amino acid uptake by lactating mammary gland *in vivo* (Viña *et al.*, 1981*a*). In order to find a possible mechanism to explain the decrease in amino acid uptake by mammary gland induced by weaning, we measured γ-glutamyltransferase activity in mammary glands of 'unilaterally weaned' rats. Mammary glands were removed from rats, chopped in fine pieces, washed three times in physiological saline (Krebs & Henseleit, 1932), to remove milk accumulated in the sealed glands, and homogenized (1:10, w/v) in Krebs–Henseleit saline.

The activity found for normal glands was 10.90 ± 1.72 μmol of *p*-nitroaniline/min per g fresh wt. (mean ± S.D.; eight observations). However, in glands with the teats sealed the γ-glutamyltransferase activity was 7.94 ± 1.56 μmol/min per g fresh wt. (mean ± S.D.; eight observations), which is significantly lower ($P < 0.005$) than that of the normal glands. However, these results must be interpreted with caution because the water content of normal and weaned glands may be different. The observed decrease in γ-glutamyltransferase activity is only 28% of the control. This may not be sufficient to explain the observed decrease in amino acid uptake by the gland. This, together with the fact that the uptake of amino acids that are not substrates for γ-glutamyltransferase is also impaired, shows that other mechanisms must exist for amino acid translocation into the lactating mammary gland. In any case, the decrease in γ-glutamyltransferase activity in 'weaned glands' provides further support to the hypothesis of the functioning of the γ-glutamyl cycle (Meister, 1973) *in vivo*.

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