Reciprocal changes in amino acid metabolism in mammary gland and liver of the lactating rat on starvation and refeeding as indicated by the tissue accumulation of α -amino[1- 14 C]isobutyrate

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Measurements of the tissue accumulation *in vivo* and *in vitro* by hepatocytes and mammary-gland acini of α -amino[1- 14 C]isobutyrate ([1- 14 C]AIB) were compared in virgin and lactating rats. The results indicate the existence of a reciprocal relationship between mammary gland and liver for AIB accumulation that is dependent on the lactational and the nutritional state of the rat. This suggests that amino acids are preferentially directed to the mammary gland during active lactation.

INTRODUCTION

The lactating mammary gland of the rat has a high requirement for amino acids to sustain the output of milk proteins. Measurements of arteriovenous differences for amino acids across the lactating gland indicate that their extraction is sensitive to food intake. Decreased accumulation is already detectable after 6 h of starvation (Viña *et al.*, 1985), and by 24 h the decrease is 80 %; refeeding with chow for 2.5 h appears to restore the arteriovenous differences to those of the fed state (Vina *et al.*, 1983). The major portion (over 50%) of the amino acids extracted by the lactating gland is accounted for by those amino acids (alanine, serine, threonine, glycine and other neutral amino acids; Viña *et al.*, 1983) transported through the cell membrane by the A system (see Collarini & Oxender, 1987). Surprisingly, in view of the high amino acid requirement of mammary gland, Casado *et al.* (1987) have reported that the hepatic extraction of the same amino acids is greatly increased during lactation. Although the increased food intake during lactation (Fell *et al.*, 1963) will provide more substrate, the reported high rates of amino acid extraction by liver and mammary gland suggest that competition for the available amino acids between the two tissues may occur and that this may be dependent on the dietary state. However, the measurements of amino acid extraction by liver and mammary gland were made in anaesthetized rats, and involve a degree of surgical trauma and certain assumptions about tissue blood flow. To examine this question of competition between liver and mammary gland by a non-invasive approach (see also Argilés *et al.*, 1989), we have used the tissue accumulation *in vivo* of α -amino[1- 14 C]isobutyrate ([1- 14 C]AIB), a non-metabolizable analogue of alanine. The tissue distribution of AIB was compared in virgin and lactating rats and the effects of starvation and refeeding were also studied. In addition, measurements of AIB accumulation have been made in isolated hepatocytes and mammary-gland acini.

EXPERIMENTAL

Animals

Wistar rats were maintained at 22 ± 2 °C in a 12 h-light/-dark cycle (light from 07:00 h) and were allowed free access to water and a stock diet consisting of 52 % carbohydrate, 21 % protein

and 4 % fat, the residue being non-digestible material (Special Diet Services, Witham, Essex, U.K.). Lactating rats had 8–10 pups and were used 10–12 days *post partum*. Female virgin rats weighed 200 ± 5 g.

Chemicals

[1- 14 C]AIB was obtained from Amersham International, Amersham, Bucks., U.K. Non-radioactive AIB and other chemicals were obtained from Sigma Chemical Co., Poole, Dorset, U.K.

Treatment of rats

All experiments started at 09:00 h. Starved rats had food withdrawn for 24 h. Pups were allowed to suckle throughout the experiment unless otherwise stated. Some lactating weaned rats had their litters removed 24 h earlier. To examine the effects of refeeding, starved rats were refed with 5 g of chow and then injected with [1- 14 C]AIB 10 min before being killed. In some experiments, conscious unrestrained rats were treated by infusion with 0.9 % NaCl containing 1 % BSA or insulin (120 m-units/h per kg; Actrapid, Novo, Denmark) in 0.9 % NaCl with 1 % albumin via jugular-vein cannulae (see below) for 1 h, or by intraperitoneal injection with 0.9 % NaCl or glucagon (25 μ g in 0.5 ml of NaCl) 2 h before being killed. The low dose of insulin did not cause marked hypoglycaemia (Jones *et al.*, 1984).

Measurement of [1- 14 C]AIB accumulation *in vivo*

Rats had polyethylene cannulae (internal diameter 0.63 mm; external diameter 0.96 mm) inserted into the left jugular vein under anaesthesia (Jones *et al.*, 1984); 3 days later, when food intake and litter-weight gain had returned to normal, the rats were used in the experiments. A weighed dose of [1- 14 C]AIB (2 μ Ci in 0.4 ml of 0.9 % NaCl) followed by 0.5 ml of 0.9 % NaCl was injected into conscious rats via the jugular-vein cannula. Only trace amounts of [1- 14 C]AIB were injected, and a short time of exposure was used to test for putative rapid changes in tissue accumulation on refeeding. More than 90 % of the injected dose had left the plasma within 10 min (Table 1). Arterial and mammary-gland venous blood samples were taken into heparinized syringes 10 min later (2–3 min after induction of anaesthesia with pentobarbital), and tissues were rapidly excised from the rat and freeze-clamped in liquid N₂ (Wollenberger *et al.*, 1960). The

Measurement of AIB accumulation by mammary-gland acini *in vitro*

Measurement of AIB accumulation by hepatocytes *in vitro*

RESULTS AND DISCUSSION

Distribution of AIB *in vivo*

In the virgin rat, the mammary-gland accumulation of AIB on a per-g basis was about 25 % of that of liver (Table 1). Starvation for 24 h decreased this value to 12 %, mainly due to an increase in accumulation by the liver (Table 1). In contrast with liver, heart and skeletal muscle showed decreased accumulation of AIB on starvation. At peak lactation (10–12 days) the AIB accumulation by mammary gland on a per-g basis was 4-fold

Table 1. Effects of starvation and refeeding on tissue accumulation of AIB in virgin and lactating rats

For full details see the Experimental section. Tissue AIB accumulation is expressed both as a percentage of the injected dose per g of tissue or per ml of plasma, and also as the tissue/plasma ratio. The percentage of injected dose remaining in total plasma was calculated by assuming that the plasma represents 4% of the body wt. The results are mean values $\pm$ S.E.M., with the numbers of animals indicated in parentheses. Values that are significantly different by Student's *t* test from the corresponding value for fed virgin rats are indicated by **P* < 0.05, ***P* < 0.01, ****P* < 0.001. Values for lactating rats that are significantly different from fed lactating rats are indicated by †*P* < 0.05, †††*P* < 0.001.

Tissue	Experimental group									
	Virgin, fed (7)		Virgin, starved (24 h)		Lactating, fed (9)		Lactating, starved (24 h)		Lactating, re-fed (6 h)	
	(% of injected dose/g)	(tissue/plasma)	(% of injected dose/g)	(tissue/plasma)	(% of injected dose/g)	(tissue/plasma)	(% of injected dose/g)	(tissue/plasma)	(% of injected dose/g)	(tissue/plasma)
Mammary gland	0.27 ± 0.01	0.44 ± 0.04	0.23 ± 0.01*	0.38 ± 0.04	1.13 ± 0.13***	4.55 ± 0.55***	0.53 ± 0.03***†††	1.32 ± 0.16***†††	0.83 ± 0.08***	2.67 ± 0.25***†
Liver	1.14 ± 0.08	1.73 ± 0.12	1.98 ± 0.14***	2.94 ± 0.49*	0.29 ± 0.03***	1.23 ± 0.12*	0.99 ± 0.11***†††	2.42 ± 0.24***†††	0.50 ± 0.04***††††	1.61 ± 0.23
Heart	0.34 ± 0.04	0.49 ± 0.02	0.18 ± 0.01**	0.31 ± 0.03***	0.16 ± 0.02***	0.63 ± 0.05*	0.14 ± 0.02***	0.31 ± 0.04***†††	0.21 ± 0.03*	0.64 ± 0.08
Skeletal muscle	0.42 ± 0.11	0.24 ± 0.03	0.14 ± 0.01*	0.22 ± 0.02	0.10 ± 0.04**	0.38 ± 0.07	0.09 ± 0.01**	0.24 ± 0.04	0.10 ± 0.01*	0.29 ± 0.04
Total plasma	4.85 ± 0.37	—	5.00 ± 0.37	—	2.93 ± 0.17***	—	3.89 ± 0.30†	—	3.09 ± 0.41*	—

Table 2. Accumulation of AIB by isolated hepatocytes from virgin and lactating rats and isolated mammary-gland acini from lactating rats

For full details see the Experimental section. AIB accumulation is expressed as nmol/min per g fresh wt. of tissue. The fresh wt. of acini was calculated from the DNA values by using the DNA contents of fresh mammary tissue for fed and starved lactating rats reported by McNeillie & Zammit (1982). The results are mean values $\pm$ S.E.M., with the numbers of experiments in parentheses. Values that are significantly different by Student's *t* test from the corresponding fed value are indicated by **P* < 0.05, ***P* < 0.01.

Preparation	Experimental group			
	Virgin rats		Lactating rats	
	Fed	Starved (24 h)	Fed	Starved (24 h)
Hepatocytes	65.1 $\pm$ 4.2 (5)	59.6 $\pm$ 1.7 (4)	29.4 $\pm$ 2.3 (6)	45.0 $\pm$ 4.3 (5)**
Mammary-gland acini	—	—	123.5 $\pm$ 16.2 (9)	76.5 $\pm$ 12.8 (8)*

higher than that of mammary tissue of virgin rats, whereas the liver accumulation in lactating rats was only 25% of that in virgin rats (Table 1). The apparent predominance of the lactating mammary gland as a site of AIB removal is even more pronounced if the AIB accumulation (percentage of the injected dose) is expressed on a total-tissue basis (virgin: liver 10.3%, mammary gland 1.1%; lactating: liver 4.2%, mammary gland 20.4%). Thus the total accumulation of AIB in mammary gland plus liver was 11.4% of the injected dose in virgin rats and 24.6% in lactating rats. This increase in lactation is partly accounted for by decreased accumulation into muscle and increased removal from the plasma (Table 1). Removal (24 h) of the suckling pups decreased the direction of AIB to the mammary gland and enhanced liver accumulation (results not shown).

Starvation (24 h) of lactating rats resulted in a 50% decrease in accumulation of AIB by lactating mammary gland on a per-g-wt. basis (Table 1), corresponding to 5.8% of the injected dose on a total-tissue basis. This decrease (about 80%) on starvation is in good agreement with the decrease in total amino acid extraction measured by arteriovenous differences across the gland (Viña *et al.*, 1983, 1985, 1987). Interestingly, there was a reciprocal change in liver accumulation of AIB on starvation, which increased 3-fold on a per-g-wet-wt. basis (Table 1) and approx. 2-fold on a total-tissue basis (9.9% of injected dose), so that liver was now the predominant tissue of AIB removal. A number of the processes in the lactating gland, including glucose transport (Threadgold & Kuhn, 1984; Bussmann *et al.*, 1984), lactose synthesis (Carrick & Kuhn, 1978) and lipogenesis (Robinson *et al.*, 1978; Bussmann *et al.*, 1984), are depressed by starvation and are rapidly restored on refeeding. To examine whether the same is true of AIB accumulation, starved (24 h) lactating rats were fed on 5 g of chow diet for various times (0.5, 1, 2 and 6 h) and the tissue distribution of AIB was measured. Only the 6 h value for the mammary tissue was significantly increased (60%) above the starved value; there was a parallel decrease (50%) in the hepatic AIB accumulation (Table 1). Insulin infusion for 60 min into starved lactating rats did not significantly alter AIB accumulation into mammary gland or liver (results not shown). Glucagon administration to fed lactating rats did not alter mammary-gland AIB accumulation, but increased accumulation to the starved-lactating-liver value ($1.00 \pm 0.25\%$ of absorbed dose/g; *n* = 6; *P* < 0.01). Mammary gland has no glucagon receptors (Robson *et al.*, 1984), and this explains the absence of an effect in this tissue. The decreased accumulation of AIB on starvation by the mammary gland might be due in part to the decreased (40–50%) blood flow (Jones & Williamson, 1984; Viña *et al.*, 1985), which does not return to the fed value within 2 h of refeeding (Jones & Williamson, 1984). To examine the possibility that entry of AIB

is altered in liver and mammary gland on starvation, irrespective of blood flow, we have measured accumulation *in vitro*.

AIB accumulation *in vitro*

In contrast with the findings *in vivo* (Table 1), AIB accumulation by hepatocytes from virgin rats was not altered by starvation (24 h) under our conditions *in vitro* (Table 2). Others have demonstrated with lower AIB concentrations (0.1 mM) the emergence of a high-affinity transport system in isolated hepatocytes on more prolonged starvation (48–72 h) (Fehlmann *et al.*, 1979; Caro & Amatruda, 1980). An extensive study by Bellemann (1981) indicated that hepatic AIB accumulation *in vitro* increased progressively with length of starvation. Hayes & McGivan (1982) found that starvation (48 h) increased (80–90%) alanine (> 1 mM) accumulation by hepatocytes; this increase was similar to that reported for AIB (0.1 mM) (Caro & Amatruda, 1980), but considerably lower than that reported by Fehlmann *et al.* (1979). Hepatocytes from fed lactating rats showed a decreased (about 50%) accumulation of AIB compared with those from virgin rats, and the rate increased (53%) on starvation of lactating rats (Table 2), which parallels the findings *in vivo* (Table 1). The decreased accumulation of AIB by hepatocytes from fed lactating rats is in agreement with a similar decrease (30%) in alanine removal (Viña & Williamson, 1981). However, neither these findings *in vitro*, nor the results *in vivo* (Table 1), agree with the report by Casado *et al.* (1987) that alanine extraction by the lactating liver *in vivo* is greatly increased.

Under the same incubation conditions, the accumulation of AIB by mammary-gland acini was 4 times that by isolated hepatocytes from the same group of rats (Table 2); this difference in accumulation *in vitro* between the two tissues is in good agreement with that seen *in vivo* (Table 1). Starvation (24 h) decreased (40%) the AIB accumulation by isolated acini, and it was no longer significantly different from that by isolated hepatocytes from starved rats (Table 2). A similar decrease in AIB accumulation by mammary-gland acini on starvation has been reported by Viña *et al.* (1987). Addition of insulin (50 m-units/ml) to the incubation medium did not significantly increase AIB accumulation in acini from starved lactating rats.

Conclusions

These experiments indicate that there is a reciprocal relationship between mammary-gland and liver accumulation of AIB in lactation, and that this is dependent on the food intake. If it is assumed that AIB accumulation is representative of alanine and other amino acids transported by the A system, this reciprocal relationship makes excellent physiological sense. The outstanding questions are the mechanisms and signals which bring about the changes. The present evidence indicates that changes in AIB

accumulation in mammary gland during the fed-starved transition are in part due to alterations in blood flow, but the experiments *in vitro* suggest that, in addition, in mammary gland and liver there are changes in the capacity to accumulate AIB. Insulin does not appear to stimulate rapidly AIB accumulation into lactating mammary gland, but the decreased insulin/glucagon ratio in starvation (Robinson *et al.*, 1978) may be responsible for the increased hepatic accumulation of AIB.

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