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# Hepatic uptake of amino acids at mid-lactation in the rat

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Hepatic availability and uptake of amino acids were measured in fed virgin and 15-day-lactating rats. Lactation did not induce any change in total amino acid availability (expressed per 100 g body wt.). Virgin rats showed a nil hepatic balance, and lactation induced a high net uptake. The high drainage of amino acids by mammary gland does not affect hepatic availability.

## INTRODUCTION

There is now strong evidence supporting the view that the mammary gland is a main site of amino acid metabolism at the peak of lactation (Williamson, 1986). This has been assessed either by measuring the amino acid arteriovenous differences (J. Viña *et al.*, 1981; Viña & Williamson, 1981*b*) or by incubating acini isolated from mammary glands (Viña & Williamson, 1981*a*,*b*). The involvement of the γ-glutamyl-transpeptidase cycle in mediating amino acid uptake is well known (J. Viña *et al.*, 1981), as well as the main metabolic fates of these compounds in the mammary gland: synthesis of milk proteins and lipogenesis (Viña & Williamson, 1981*a*,*b*).

However, as Williamson (1986) pointed out, there is little information about amino acid metabolism in tissues other than the mammary gland. Since lactating rats are hyperphagic (Fell *et al.*, 1963), it has been suggested that the eventual excess of dietary amino acids should be metabolized by the mammary gland and the liver. On the other hand, there is no evidence of the onset of any nitrogen-sparing mechanism during this period, in contrast with what happens during pregnancy (Freinkel *et al.*, 1972).

In order to establish the role of the liver in amino acid metabolism during lactation, we have quantified the hepatic amino acid availability, uptake and fractional extraction in virgin and 15-day-lactating rats.

## MATERIALS AND METHODS

### Animals

Virgin rats of the Wistar strain bred in our laboratory were used. They were mated at 65 days of age (200 g body wt.) and used for experiments on day 15 after delivery. Litters were randomized to ten pups. Animals were caged individually and maintained under controlled conditions of light (12 h on/12 h off light cycle) and temperature (20±1 °C). They were fed *ad libitum* on a laboratory chow (Panlab, Spain) of the following composition: protein, 17.2% (50% high-biological-quality animal protein); lipid, 3.5%; fibre, 4.5%; free sugars, 4.1%; starch, 45%; minerals, 4.5%.

### Experimental design

The 15-day-lactating rats and virgin controls were each randomly divided into two subgroups to carry out two different sets of experiments. The first set was performed to measure the portal and hepatic blood flows. In the second, we estimated the arteriovenous substrate differences across the liver. All the experiments were performed 150 min after the beginning of the light cycle.

### Blood-flow procedure

For measurement of portal and hepatic blood flows the indicator-dilution method was used, with *p*-aminohippuric acid (Katz & Bergman, 1969). Rats were anaesthetized with sodium pentobarbital (60 mg/kg body wt., intraperitoneally). After laparotomy, *p*-aminohippuric acid (0.02% in saline) was infused in a mesenteric vein at a rate of 0.09 ml/min after the administration of a priming dose of 0.2 mg. After 4 min infusion, blood was slowly sampled in less than 55 s from the hepatic and portal veins and then from the aorta. Under these conditions, haemorrhage and hypovolaemic shock were avoided, and haematocrit values remained unchanged (Pastor-Anglada *et al.*, 1987). Arterial steady-state concentrations of the indicator were achieved before 4 min infusion in either lactating or virgin rats, as assessed in a previous set of experiments on rats provided with indwelling arterial catheters and infused with *p*-aminohippuric acid in a mesenteric vein (results not shown). Blood samples were deproteinized by adding $HClO_4$ (final concn. 5%, w/v). Supernatants were used for the *p*-aminohippuric acid assay as previously described (Desbordes & Samorcq, 1963). Blood from non-infused animals was supplemented with known amounts of *p*-aminohippuric acid and used as a standard.

### Substrate determinations

Extraction and deproteinization of blood samples from virgin and lactating rats were done as described above. Free amino acids were measured in the protein-free extracts by using an amino acid analyser, as previously reported (Pastor-Anglado & Remesar, 1986).

### Calculations

Substrate availability ($A$) was calculated as follows:

$$A = (F_p \times C_p) + (F_a \times C_a)$$

where $F_p$ and $F_a$ represent the portal and hepatic-artery blood flows, and $C_p$ and $C_a$ the substrate concentrations in portal vein and aorta respectively. Substrate uptake ($U$) by the liver was determined as:

$$U = A - (F_h \times C_h)$$

where $F_h = F_p + F_a$, and $C_h$ is the substrate concentration in the hepatic vein. Substrate fractional extraction (FE) was calculated as follows:

$$FE = (U/A) \times 100$$

### Statistics

All results are expressed as means ± S.E.M. Significance of changes induced by lactation was tested by Student's $t$ test.

## RESULTS

### Body and liver weights

Body weights of lactating and virgin rats were 293.6 ± 9.14 and 206.9 ± 6.6 g respectively. Liver weights were 13.35 ± 0.42 g in lactating rats and 8.35 ± 0.27 g in virgin rats.

### Blood flows

Total hepatic blood flow was not affected by lactation (34.8 ± 3.1 ml/min for lactating rats and 33.3 ± 3.0 ml/min for virgin rats), but it was significantly decreased when corrected by liver weight (2.6 ± 0.3 and 4.0 ± 0.4 ml/min per g of liver respectively; $P < 0.05$). This difference was due to a lower hepatic-artery blood flow, since portal flow remained unchanged (2.0 ± 0.1 and 2.2 ± 0.2 ml/min per mg of liver for lactating and virgin rats respectively).

### Arterial concentrations

Table 1 shows amino acid concentrations (μM) in aorta. Lactation did not affect essential amino acid concentrations. As regards the non-essential amino acids, lactation induced lower concentrations of taurine and higher values for alanine, glycine, serine, glutamate, asparagine and ornithine.

### Hepatic availability

Hepatic availability of amino acids, expressed as μmol reaching the liver/min per 100 g body wt., is shown in Table 2. Although there was no difference in the availability of essential amino acids when considered as a whole, lactation induced a decrease in the availabilities of tryptophan and lysine. In lactating rats, there were also decreases in the availabilities of taurine, glutamine, citrulline and arginine, and increases in those of serine, asparagine, glycine and alanine.

### Hepatic uptake

Amino acid uptake by the liver, expressed as μmol taken up/min per 100 g body wt., is shown in Table 3.

Lactation induced a very high hepatic uptake of individual amino acids. Only methionine, tryptophan, glutamine, ornithine and arginine did not present an uptake significantly different from zero. All the other amino acids showed a net uptake by the liver, statistically different from the values found in virgin controls (except for taurine, aspartate, asparagine, glutamate and tyrosine).

**Table 1. Arterial concentrations of amino acids (μM)**

Statistical differences: lactating versus virgin, $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

| Amino acid | Rats... / No. of animals... | Concn. (μM) Virgin 10 | Concn. (μM) Lactating 7 |
|---|---|---|---|
| Threonine | | 210 ± 14.9 | 207 ± 21.4 |
| Methionine | | 18.1 ± 4.5 | 12.6 ± 4.1 |
| Valine | | 128 ± 11.4 | 113 ± 16.8 |
| Isoleucine | | 53.5 ± 4.5 | 54.1 ± 5.3 |
| Leucine | | 94.1 ± 10.3 | 83.7 ± 8.4 |
| Phenylalanine | | 41.0 ± 2.8 | 45.1 ± 4.9 |
| Tryptophan | | 21.3 ± 4.6 | 13.5 ± 1.6 |
| Lysine | | 483 ± 23.7 | 428 ± 65.9 |
| Total essential | | 1068 ± 65.8 | 981 ± 120 |
| Taurine | | 283 ± 13.8 | 220 ± 9.1** |
| Serine | | 158 ± 7.2 | 198 ± 15.8* |
| Aspartate | | 39.4 ± 1.8 | 47.3 ± 4.0 |
| Asparagine | | 45.6 ± 3.1 | 63.2 ± 4.1** |
| Glutamate | | 116 ± 6.3 | 147 ± 13.4* |
| Glutamine | | 612 ± 34.3 | 666 ± 81.5 |
| Proline | | 161 ± 27.2 | 161 ± 11.5 |
| Glycine | | 194 ± 9.4 | 243 ± 10** |
| Alanine | | 358 ± 31.9 | 517 ± 57.4* |
| Citrulline | | 89.2 ± 6.4 | 87.6 ± 4.8 |
| Tyrosine | | 29.6 ± 3.2 | 34.6 ± 2.4 |
| Histidine | | 61.7 ± 2.8 | 59.9 ± 2.5 |
| Ornithine | | 62.3 ± 4.5 | 88.1 ± 9.9* |
| Arginine | | 206 ± 17.1 | 189 ± 22.8 |
| Total amino acids | | 3592 ± 158 | 3732 ± 273 |

**Table 2. Hepatic availability of amino acids**

Statistical differences are indicated as in Table 1.

| Amino acid | Rats... / No. of animals... | Hepatic availability (μmol reaching the liver/min per 100 g body wt.) Virgin 10 | Hepatic availability (μmol reaching the liver/min per 100 g body wt.) Lactating 7 |
|---|---|---|---|
| Threonine | | 3.17 ± 0.25 | 3.10 ± 0.12 |
| Methionine | | 0.28 ± 0.06 | 0.14 ± 0.02 |
| Valine | | 1.93 ± 0.14 | 1.75 ± 0.11 |
| Isoleucine | | 0.91 ± 0.06 | 0.94 ± 0.10 |
| Leucine | | 1.48 ± 0.12 | 1.47 ± 0.13 |
| Phenylalanine | | 0.67 ± 0.04 | 0.68 ± 0.06 |
| Tryptophan | | 0.39 ± 0.06 | 0.21 ± 0.03* |
| Lysine | | 7.51 ± 0.25 | 5.66 ± 0.16*** |
| Total essential | | 16.1 ± 0.80 | 14.1 ± 0.34 |
| Taurine | | 5.22 ± 0.32 | 3.10 ± 0.30*** |
| Serine | | 2.39 ± 0.13 | 2.80 ± 0.13* |
| Aspartate | | 0.71 ± 0.06 | 0.73 ± 0.06 |
| Asparagine | | 0.83 ± 0.06 | 1.30 ± 0.04*** |
| Glutamate | | 2.14 ± 0.10 | 2.26 ± 0.07 |
| Glutamine | | 8.26 ± 0.46 | 6.89 ± 0.29* |
| Proline | | 3.30 ± 0.48 | 2.67 ± 0.13 |
| Glycine | | 3.43 ± 0.14 | 4.47 ± 0.32** |
| Alanine | | 7.19 ± 0.72 | 9.46 ± 0.42* |
| Citrulline | | 1.65 ± 0.14 | 1.21 ± 0.07* |
| Tyrosine | | 0.47 ± 0.04 | 0.51 ± 0.03 |
| Histidine | | 1.00 ± 0.04 | 0.96 ± 0.05 |
| Ornithine | | 1.05 ± 0.06 | 1.15 ± 0.05 |
| Arginine | | 3.34 ± 0.24 | 2.37 ± 0.12* |
| Total amino acids | | 56.8 ± 1.9 | 53.9 ± 2.0 |

**Table 3. Hepatic uptake of amino acids**

Statistical differences: lactating versus virgin, $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Lactating or virgin versus zero, $\dagger P < 0.05$; $\dagger\dagger P < 0.01$; $\dagger\dagger\dagger P < 0.001$.

| Amino acid | Rats... No. of animals... | Uptake ($\mu$mol/min per 100 g body wt.) Virgin 10 | Lactating 7 |
|---|---|---|---|
| Threonine | | 0.01±0.10 | 0.56±0.05**††† |
| Methionine | | −0.14±0.11 | 0.01±0.02 |
| Valine | | −0.11±0.11 | 0.30±0.07*†† |
| Isoleucine | | 0.04±0.03 | 0.17±0.02*†† |
| Leucine | | 0.09±0.04 | 0.32±0.05**†† |
| Phenylalanine | | 0.08±0.02†† | 0.22±0.03**†† |
| Tryptophan | | 0±0.02 | −0.02±0.02 |
| Lysine | | 0.08±0.23 | 0.98±0.32*† |
| Total essential | | −0.50±0.45 | 2.94±0.53***†† |
| Taurine | | 0.71±0.29† | 0.88±0.28† |
| Serine | | 0±0.08 | 0.53±0.06***††† |
| Aspartate | | 0.03±0.06 | 0.14±0.03†† |
| Asparagine | | 0.27±0.04††† | 0.36±0.03††† |
| Glutamate | | 0.03±0.09 | 0.22±0.06† |
| Glutamine | | −0.46±0.22 | −0.75±0.43 |
| Proline | | 0.93±0.54 | 0.70±0.15†† |
| Glycine | | 0.51±0.12†† | 1.58±0.15***††† |
| Alanine | | 1.60±0.26††† | 4.32±0.35***††† |
| Citrulline | | −0.03±0.07 | 0.26±0.03**††† |
| Tyrosine | | 0.11±0.04† | 0.20±0.01††† |
| Histidine | | 0.09±0.02†† | 0.23±0.03**††† |
| Ornithine | | −0.08±0.06 | 0±0.04 |
| Arginine | | 0.16±0.12 | 0.17±0.20 |
| Total amino acids | | 1.72±0.92 | 11.5±1.40***††† |

These facts were reflected in higher hepatic balances either for the whole essential amino acids or for the whole amino acids, these balances being significantly different from zero and from the balances found in virgin rats.

### Fractional extraction

Hepatic fractional extraction was statistically different from zero in those cases in which positive hepatic uptakes were found. Thus the livers of 15-day-lactating rats showed higher fractional extractions for essential amino acids (21.6±4.0 versus 0.2±1.8%; $P < 0.001$) (especially for threonine, valine, isoleucine, leucine and phenylalanine) and for the whole amino acids (19.2±1.6 versus 3.3±1.5%; $P < 0.001$), owing to the higher fractional extractions for serine, glycine, alanine, citrulline, tyrosine and histidine.

## DISCUSSION

The indicator-dilution method for measuring hepatic blood flows has been adapted to small laboratory animals such as the rat (Rémésy & Demigné, 1983). Our results for virgin controls are slightly higher than those previously reported by others using the same procedure (Rémésy *et al.*, 1983). Hepatic blood flow in mid-lactating rats is, however, lower than that reported by Hanwell & Linzell (1973), who used a method based on $^{86}$RbCl distribution.

The indicator-dilution technique allows direct measurements of hepatic blood flow, but when adapted to small rodents implies the use of anaesthesia. However, pentobarbital anaesthesia does not seem to alter the hepatic blood flow in adult non-lactating rats, as reported by using radiolabelled microspheres (James *et al.*, 1986). Furthermore, it has also been proved that mammary-gland blood flow does not significantly change after pentobarbital anaesthesia (Jones & Williamson, 1984; Mercer & Williamson, 1986).

The nil net hepatic uptake of most amino acids in virgin rats is in agreement with previous reports (Pastor-Anglada & Remesar, 1986; Casado *et al.*, 1986) and may be the consequence of rats being in a near post-absorptive state. In fact, even in absorptive conditions (4 h after the beginning of the daily food intake), rats fed with a hyperglucidic diet showed low portal−hepatic-vein differences and fractional extractions for most amino acids (Rémésy *et al.*, 1978). In spite of this finding, the liver of 15-day-lactating rats retained a large quantity of incoming amino acids, either essential or not. Under these nutritional conditions, the mammary gland is also taking up a considerable amount of amino acids (Viña *et al.*, 1983); thus it is suggested that amino acid metabolism of mid-lactation should be very active, and an endogenous and/or an exogenous source would support the high requirements of these two organs. Although it appears that the excess of dietary amino acids would be enough to sustain the requirements of liver and mammary gland, since food intake is increased during lactation (Fell *et al.*, 1963), we found that hepatic availability corrected by body weight did not differ between lactating and virgin rats. It has been reported, in either fed or starved rats, that the net hepatic balance for some amino acids with different transport specificities seems to be directly correlated with portal amino acid availability (Taylor & Rennie, 1986). In our experiments, when we plotted the mean hepatic availability for each amino acid against its own uptake (results not shown), we found a high correlation in lactating ($r = 0.67$, $n = 22$; $P < 0.01$), but not in virgin, rats ($r = 0.34$, $n = 22$). This means that the higher hepatic uptake of amino acids is not a mere function of their availabilities, but rather the consequence of a greater capability to take up these compounds, as reflected by the fractional extraction rates.

Previous reports (Fafournoux *et al.*, 1983; Pastor-Anglada *et al.*, 1987) showed that alterations in hepatic fractional extraction rates may be accompanied by changes in the hepatocyte transport-system kinetics, and so the fractional extraction rate may reflect approximately the hepatic transport-system capacity that would be increased during lactation. However, to what extent amino acid transport kinetics determines the net uptake of amino acids *in vivo* is still a matter of controversy.

When considering individual amino acids, it becomes obvious that the increase in whole amino acid uptake during lactation is due to a generalized increase in the uptake of most of them. The increase in the uptake of some of the potentially more gluconeogenic amino acids, such as serine, glycine and alanine, is particularly noticeable. This increase is mediated by two factors, a high fractional extraction and a high hepatic availability. Thus the arterial-blood concentrations of serine, glycine and alanine were significantly increased. This is a general trend of mid-lactation, which agrees with previous reports (J. R. Viña *et al.*, 1981).

However, these results disagree with the work of Viña & Williamson (1981*b*), who indicated that alanine utilization by isolated hepatocytes from fed mid-lactating rats is decreased compared with fed virgins. These authors suggested that alanine-utilization rates on a cell-number basis may differ from those expressed on a whole-liver basis. Moreover, alanine availability *in vivo* is higher in mid-lactating than in virgin rats. The low insulin/glucagon ratio (Burnol *et al.*, 1983; Williamson, 1986) may also modulate hepatic amino acid uptake *in vivo* at mid-lactation. Glucagon exerts an stimulatory effect on A- and ASC-system-mediated amino acid transport by a rapid and protein-synthesis-independent mechanism, which implies membrane hyperpolarization (Moule *et al.*, 1987). This effect, acting *in vivo*, could be lost after hepatocyte isolation.

Our results make it clear that the great drainage of amino acids by the mammary gland does not affect hepatic availability of these substrates. Moreover, in spite of the high requirements of the mammary gland, the livers of lactating rats showed an increased uptake of amino acids. In conclusion, this experiment does not support the existence of any nitrogen-sparing mechanism during lactation at the amino acid inter-organ-relationship level.

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