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Glutamine as a major nitrogen carrier to the liver in suckling rat pups

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We measured the amino acid concentrations in the afferent and efferent vessels of the liver in anaesthetized fed adult rats and in fed suckling rat pups. A much higher content of glutamine in the portal vein and the aorta than in hepatic veins suggests that this amino acid is actively taken up by the liver of fed suckling rat pups, conversely to what is found in adult rats. In an attempt to characterize further the mechanism(s) contributing to this enhanced glutamine uptake, we monitored the time course of 1 mM-glutamine transport into plasma-membrane vesicles purified from the livers of either adult or suckling rats. The concentrative Na^+-dependent uptake of glutamine was lower in those vesicles obtained from pups than in those obtained from adult rats. Glutaminase and glutamine synthetase activities in livers from both experimental groups were also measured. Glutaminase and glutamine synthetase activities in suckling rats were about 3-fold higher and 2-fold lower respectively than those in adult rats. It is concluded that glutamine is a main nitrogen carrier to the liver in fed suckling rats. A high availability of this amino acid and an enzyme imbalance between glutamine-synthesizing and -degrading activities may account for the net uptake found *in vivo*.

INTRODUCTION

The main features of amino acid metabolism during the fetal and postnatal life of mammals have been reviewed by Snell (1982), Jones & Rolph (1985) and Remesar *et al.* (1987). During this period, the hormonal and nutritional status is changing rapidly and the liver is undergoing important metabolic adaptations until reaching, after weaning, most of its adult characteristics.

During suckling there is a high hepatic protein accretion which seems to be caused by means of a lowering of protein-degradation rates rather than by increasing its synthesis (Conde & Scornik, 1977; Russell *et al.*, 1980). Furthermore, most of the amino acid-degrading enzymes have low activities and, in some species, the ureagenic flux is much lower than that of adult animals (Kadowaki *et al.*, 1983). Although the protein content of milk is relatively low, 25% of the total energy of rat milk is given as protein (Ferré *et al.*, 1986). At the same time, the Na^+-dependent capacity of the liver to take up α-aminoisobutyric acid is enhanced during suckling and decreases as weaning approaches (Bellemann, 1981*a*,*b*). All these findings suggest that the amino acid supply to the liver and even their net uptake may be different from what is known to occur in the adult. In the present work we have studied this specific point by measuring the hepatic afferent and efferent concentrations of amino acids. Our results show that glutamine is the main nitrogen carrier to the liver during suckling. Two other sets of experiments have been designed in order to explain how this unusual net glutamine uptake is mediated.

MATERIALS AND METHODS

Animals

Adult rats (200 g body wt.) and 15-day suckling rat pups of the Wistar strain were used. Litters were randomized to ten pups at delivery and kept with their mothers until surgery or death. Animals were 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 (by wt.): 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

Three different sets of experiments were carried out 150 min after the beginning of the light cycle. The first was performed to measure the amino acid concentrations in blood in the respective vessels. In the second we estimated the time course of L-glutamine transport in liver plasma-membrane vesicles from both experimental groups. The third set was aimed at the measurement of glutamine synthetase and glutaminase activities in livers from both adult and suckling rats.

Surgery, blood sampling and substrate determinations

Adult and suckling rats were anaesthetized with sodium pentobarbital (60 mg/kg body wt., intraperitoneally). In adult rats, blood was slowly sampled in less than 55 s from the hepatic and portal veins and then from the aorta, as previously described (Pastor-Anglada *et al.*, 1987). In every suckling rat, blood was sampled only from one vessel in order to avoid the eventual hypovolaemic shock induced by repetitive sampling from the same animal. Processing of samples and amino acid determinations in blood were performed as previously reported (Casado *et al.*, 1987*a*).

Preparation of plasma-membrane vesicles

Plama-membrane vesicles from livers of both adult and suckling rats were prepared as previously reported

(Pastor-Anglada *et al.*, 1987). Protein in homogenate and membrane preparations was determined as described by Peterson (1979). 5'-Nucleotidase (EC 3.1.3.5) activity was selected as a plasma-membrane marker, and was assayed in both fractions as described by Aronson & Touster (1974). Three enzyme markers of membranes pertaining to other subcellular organelles were assayed in both homogenate and plasma-membrane preparations: cytochrome oxidase (EC 1.9.3.1) (mitochondrial marker) as described by Rafael (1983), β-*N*-acetylglucosaminidase (EC 3.2.1.30) (lysosomal marker) as described by Carroll (1978), and glucose-6-phosphatase (EC 3.1.3.9) (marker of endoplasmic reticulum) as described by de Duve *et al.* (1955).

Transport assays

The time course of glutamine transport was determined by a filtration procedure adapted from that of Sips *et al.* (1980). A 20 μl portion of a plasma-membrane preparation was mixed with 80 μl of an incubation mixture to give the following concentrations: 0.25 M-sucrose, 0.2 mM-$CaCl_2$, 10 mM-$MgCl_2$, 10 mM-Hepes/KOH, pH 7.4, 100 mM of either sodium or potassium sulphocyanate and 1 mM-L-[U-^{14}C]glutamine (Amersham; sp. radioactivity 9.435 MBq/μmol). The reaction was stopped at the desired times by taking 20 μl samples of the incubation mixture and putting them into 1 ml of a cold stop solution (0.25 M-sucrose, 100 mM-NaCl, 0.2 mM-$CaCl_2$ and 10 mM-Hepes/KOH, pH 7.4). Then the whole contents of the tubes were filtered through a nitrocellulose filter (pore size 0.45 μm) and washed with 3 ml of cold stop solution, and dried filters were counted for radioactivity. Each assay was done in triplicate on pooled samples from either five adult rats or suckling rat pups from four different litters. L-Glutamine uptake was calculated from the radioactivity retained by the filters and the specific radioactivity of the medium.

Enzyme assays

Livers from adult rats and from suckling rat pups of different litters were immediately excised after decapitation and homogenized in 10 vol. of chilled 0.25 M-sucrose containing 2.5 mM-2-mercaptoethanol, with an all-glass motor-driven TenBroeck homogenizer. Crude homogenates were used for enzyme-activity measurements. Phosphate-dependent glutaminase (EC 3.5.1.2) activity was measured as described by Curthoys & Lowry (1973) (i.e. in a medium containing 0.15 M-K_2HPO_4, 20 mM-L-glutamine, 50 mM-Tris/HCl and 0.2 mM-EDTA), though modified as described by Joseph & McGivan (1978), by supplementing the medium with 2 mM-NH_4Cl, 2 mM-ATP and 10 mM-$KHCO_3$ and measuring glutamate production. Glutamine synthetase (EC 6.3.1.2) activity was determined as described by Arola *et al.* (1981), by measuring the production of γ-glutamylhydroxamate in the presence of 19.6 mM-Na_2HAsO_4 and 1.2 mM-$MnCl_2$.

Statistics

All results are expressed as means ± S.E.M. Significance of differences was tested by Student's *t* test.

RESULTS

Blood amino acid concentrations

The concentrations of total and some gluconeogenic amino acids in the respective vessels are shown in Table 1. Statistical comparisons were done in each experimental group between the amino acid concentrations in the afferent and efferent vessels. In adult rats, asparagine and

Table 1. Amino acid concentrations in blood from aorta and portal and hepatic veins

Results are means ± S.E.M. for eight to ten observations. Statistically significant differences versus hepatic-vein concentrations: $*P < 0.05$, $**P < 0.01$.

Amino acid	Rats	Concn. (μM)		
		Aorta	Portal vein	Hepatic vein
Alanine	Adult	392±46	534±61	385±63
	Suckling	285±19	324±19	312±16
Glycine	Adult	186±12	231±14*	184±13
	Suckling	344±16	356±36	289±24
Serine	Adult	168±12	151±12	159±9
	Suckling	252±15	285±24	254±15
Threonine	Adult	229±21	221±29	227±23
	Suckling	191±12	208±14	180±10
Glutamate	Adult	114±6	132±8	121±7
	Suckling	123±9	145±23	127±15
Glutamine	Adult	623±34	507±35	594±47
	Suckling	1221±176**	1181±213*	576±73
Aspartate	Adult	37±3	45±4*	34±3
	Suckling	46±3	51±3	48±3
Asparagine	Adult	47±4	60±5*	43±4
	Suckling	56±9	78±9**	37±6
Total amino acids	Adult	3477±112	3627±151	3386±161
	Suckling	4136±273*	4391±353*	3387±227

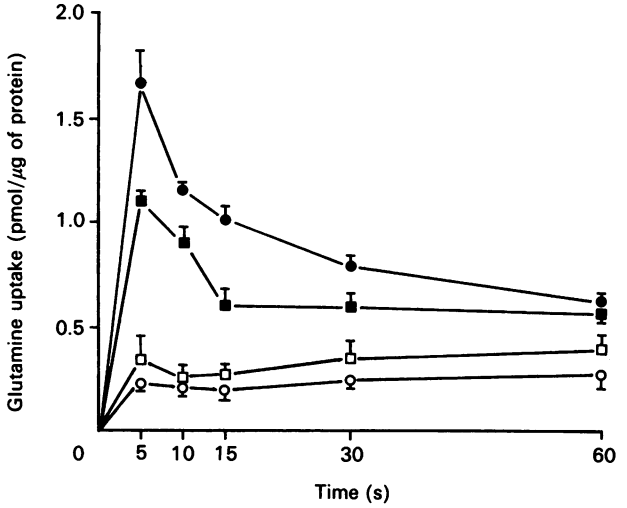

Fig. 1. Time course of glutamine uptake into liver plasma-membrane vesicles

Samples of plasma-membrane vesicles from the livers of either adult (●, ○) or suckling (■, □) rats were incubated, as indicated in the Materials and methods section, with 1 mM-glutamine in a Na^+ (●, ■) or K^+ (○, □) medium. Results are means ± S.E.M.. of three determinations on pooled samples from five animals.

glycine concentrations in the portal vein were higher than in the hepatic vein, meaning a net uptake of these amino acids by the liver of fed control animals. In suckling rat pups we also found that asparagine concentrations were higher in the portal than in the hepatic vein; nevertheless, the most significant difference was observed in the afferent concentrations of glutamine, which were much higher than those of the hepatic vein. It proved that there was an active net uptake of glutamine by the liver of fed suckling rat pups. This occurrence fully accounted for the difference between the total amino acid concentrations in afferent and efferent vessels found in this experimental group. As a general trend, the other amino acids did not show statistical differences (results not shown), and furthermore most of them usually made only a minor contribution to the hepatic amino acid uptake.

Time course of glutamine transport in plasma-membrane vesicles

It has been suggested that glutamine transport across the plasma membrane of hepatoyctes may be a regulatory site in its metabolism (Häussinger *et al.*, 1985). As an attempt to determine whether an enhanced transport activity could explain the net uptake of glutamine by the liver of suckling rats, we partially purified plasma-membrane vesicles from both experimental groups in order to study L-glutamine transport. An 8–10-fold purification was achieved for the plasma-membrane marker 5′-nucleotidase in both suckling and adult rats. Recoveries of this activity were over 11 ± 1% and 6 ± 1% for adult and suckling rats respectively. Contamination by mitochondrial and lysosomal membranes was very low, because both cytochrome oxidase and *N*-acetylglucosaminidase were recovered in low yield (0.3–0.5%) with decreased specific activities. However, recovery for glucose-6-phosphatase was over 1–2%, but with a similar specific activity.

Table 2. Liver glutamine synthetase and glutaminase activities

Activities (means ± S.E.M.) are expressed as μkat/kg of tissue. Statistically significant differences, adult versus suckling activities: †$P < 0.001$.

	Glutamine synthetase	Glutaminase	Glutaminase/ synthetase ratio
Adult	350 ± 14	8.6 ± 1.1	0.02
Suckling	174 ± 3†	10.3 ± 0.6	0.06

The time course of glutamine uptake at 5, 10, 15, 30 and 60 s in the presence of 1 mM-glutamine and either sodium or potassium sulphocyanate is shown in Fig. 1. Transport in the Na^+ medium was concentrative, but to a lesser extent in those vesicles obtained from suckling rats than in those from adult rats.

Glutaminase and glutamine synthetase activities

Glutaminase and glutamine synthetase activities in livers from both experimental groups are shown in Table 2. Hepatic glutaminase activity was slightly higher in suckling than in adult rats, although this did not reach statistical significance. Conversely, glutamine synthetase activity in liver was 2-fold higher in adults than in suckling rat pups.

DISCUSSION

Amino acid hepatic balances

Since no simultaneous measurements of blood amino acid concentrations were performed in suckling rats, we could not calculate individual hepatic arteriovenous differences. Furthermore, the calculation of hepatic balances was not possible, owing to the lack of information about portal and hepatic artery blood flows in suckling rats; the method that we previously used (Casado *et al.*, 1987*a*,*b*) was not useful in the present situation, since it implies simultaneous blood extractions from the same animal. Thus we considered the significant difference between the afferent and efferent amino acid content as a good index of a net hepatic uptake. However, the opposite consideration is not necessarily certain, and thus the lack of significance does not exclude the possibility of a net uptake. For instance, in the adult rats, portal alanine concentrations were not significantly higher than those of the hepatic vein; however, when balances were calculated individually (results not shown), we realized that there was a net and significant uptake of alanine by the liver, of the same magnitude as previously described (Rémésy *et al.*, 1978; Casado *et al.*, 1987*a*,*b*).

The finding that glutamine is taken up by the liver of suckling rat pups may have important physiological implications. A glutamine hepatic balance similar to that found in the present study has been reported in rats fed on a high-protein diet (Rémésy *et al.*, 1978), although in many other physiological situations, such as lactation (Casado *et al.*, 1987*a*) or pregnancy (Casado *et al.*, 1987*b*), rats fed on a high-carbohydrate diet (Rémésy *et al.*, 1983; Casado *et al.*, 1987*a*,*b*) or rats fasted for 24 h (Aikawa *et al.*, 1973), the hepatic balance for glutamine was not different from zero, or there was a net release of this amino acid.

Glutamine is a main oxidative substrate in the small intestine (Windmueller & Spaeth, 1980), and thus the arteriovenous differences across this organ are usually positive unless they are counterbalanced by glutamine absorbed from the intestinal tract. This is probably the case in our suckling rats, where no differences were found between glutamine concentrations in portal vein and aorta. In any case, the concentrations of this amino acid in both afferent hepatic vessels are higher in suckling than in adult rats, Thus a greater availability of glutamine may be an important factor determining its own enhanced net hepatic uptake.

Time course of L-glutamine uptake by plasma-membrane vesicles

It has been previously shown that the liver of suckling rats has an increased capacity to take up α-amino-isobutyric acid (Bellemann, 1981*a*,*b*). In order to determine if an enhanced capacity for glutamine uptake could mediate the net hepatic balance found *in vivo*, we have studied the time course of uptake of L-glutamine by liver plasma-membrane vesicles from adult and suckling rats. Our preparations had similar enzyme profiles and their concentrative uptake was higher than that reported by others (Jacob *et al.*, 1986). However, as indicated above, the overshoot at physiological concentrations of glutamine was lower in those plasma membranes isolated from suckling rats than in those from adult animals, and thus it is rather unlikely that carrier activity may be responsible for the net glutamine uptake by liver. A different pattern of development for Na^+-dependent hepatic transport systems may be possible, and may explain also the apparent discrepancy with the results of Bellemann (1981*a*,*b*). α-Aminoisobutyrate has the same mechanism as alanine for entering the hepatocyte (Kilberg, 1982). Starvation induces differential effects on alanine and glutamine transport in isolated hepatocytes, and it has been suggested that the uptake of both amino acids is controlled separately (Hayes & McGivan, 1982). This could also occur during suckling.

An eventual lower capacity of the liver glutamine carriers could be overcome by the enhanced substrate availability and by the hormonal status prevailing *in vivo*. Plasma glucagon concentrations during suckling are 2-fold greater than those found after weaning, and the insulin/glucagon ratio in suckling rats is much decreased as compared with weaned animals (Issad *et al.*, 1988). Glucagon exerts a short stimulatory effect on alanine transport by means of membrane hyperpolarization (Moule *et al.*, 1987). Because this effect seems to be mediated by changes in the Na^+ gradient, it is likely that any amino acid whose transport was Na^+-dependent (i.e. glutamine) should be under the same type of modulation.

Enzyme imbalance

In the adult liver, periportal glutaminase and perivenous glutamine synthetase catalyse the prior degradation of glutamine and its further synthesis, thus contributing to the emergence of an intercellular glutamine cycle (Häussinger, 1983). As pointed out more recently by Häussinger (1987), the relative activities of both enzymes should modulate the net hepatic uptake or release of glutamine. In 15-day suckling rats, hepatic glutaminase activity reaches values close to adult ones (Linder-Horowitz, 1969; Herzfeld & Estes, 1973), but glutamine synthetase activity is still quite low (Herzfeld & Estes, 1973). Moreover, the finding that carbamoyl-phosphate synthase I is already located periportally just after birth suggests that the liver heterogeneity of nitrogen metabolism is established at the first stages of postnatal life (Gaasbeek-Janzen *et al.*, 1985).

In our study, we show that 15-day suckling rat pups have a glutaminase/glutamine synthetase activity ratio about 3-fold greater than that found in adult rats. This enzyme imbalance should actively contribute to the net hepatic uptake found *in vivo*. In the adult liver, glutaminase activity adjusts the provision of NH_4^+ to carbamoyl-phosphate synthase I, and thus to urea synthesis (Meijer, 1985); however, this is unlikely to occur during suckling, and glutamine should be preferentially used as an NH_4^+ donor for anabolic purposes. In the perfused liver of suckling rats, the ureagenic flux has not yet reached adult values, and ammoniagenesis is increased (Snell, 1975). Flux through the urea cycle may be limited by low ornithine availability (Snell, 1982), and carbamoyl phosphate synthesized inside the mitochondria should be diverted to the cytosol for pyrimidine synthesis. This does not seem to occur in the adult liver (Pausch *et al.*, 1985), but it could be possible in the neonatal liver as far as several features are distinct. Firstly, liver carbamoyl-phosphate synthase II, the cytosolic glutamine-dependent enzyme involved in the pyrimidine pathway, shows low activities after birth (Hager & Jones, 1967). Secondly, the ureagenic flux is limited when glutamine availability and glutaminase activity are very high. Moreover, evidence of mitochondrial synthesis of carbamoyl phosphate and of its extrusion to the cytosol for pyrimidine biosynthesis has been reported in the neonatal liver by Tremblay *et al.* (1977). Besides these considerations, one should not exclude the eventual utilization of glutamine for other biosynthetic purposes, such as glycoprotein or purine synthesis (Richards & Greengard, 1973; Snell, 1982), where glutamine transaminases may play an important role in glutamine metabolism.

In conclusion, glutamine is the main nitrogen carrier to the liver in fed suckling rat pups. A high availability of this amino acid as well as an imbalance between the glutamine-synthesizing and -degrading enzyme activities could partially explain the net uptake found *in vivo*.

Glutamine modulates several metabolic fluxes and may be considered as an eventual regulator in liver, enhancing either glycogen synthesis (Katz *et al.*, 1979; Lavoinne *et al.*, 1987) or lipogenesis (Lavoinne *et al.*, 1987) in isolated rat hepatocytes. Both metabolic fluxes increase at weaning, but the eventual relationship between this phenomenon and the high glutamine uptake found in the present work requires further characterization.

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