Effect of starvation on the *N*-acetylglutamate system of rat liver

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Rats fasted for 3 and 6 days showed an increase in the activity, per g of liver but not per total liver, of the mitochondrial urea cycle enzymes, carbamylphopshate synthetase and ornithine transcarbamylase. The activity of *N*-acetylglutamate synthetase, both per g and per total liver, increased by the third day and then decreased on the sixth day of fasting. The cytosolic enzyme *N*-acetyldeacylase showed the same general pattern as the *N*-acetylglutamate synthetase except that the relative proportion of synthetase over deacylase was higher at the third day of starvation. The *N*-acetylglutamate level/g liver increases in relation to the number of days of fasting.

Carbamylphosphate synthetase *Ornithine transcarbamylase* N-*Acetylglutamate synthetase* N-*Acetylglutamate deacylase* N-*Acetylglutamate* *Arginine*

1. INTRODUCTION

The levels of the 5 main enzymes of the urea cycle change in response to various dietary and hormonal situations [1–4]. Fasting leads to increased gluconeogenesis from amino acids obtained by proteolysis and is associated with increased activity of the urea cycle and with high levels of the 5 main enzymes [1].

In the last few years it has become evident that *N*-acetylglutamate plays a key role in the regulation of urea biosynthesis [5–8]. The levels of this essential activator of carbamylphosphate synthetase [9] change in response to nitrogen load and in part control the rate of urea production [1,6,10]. The level of *N*-acetylglutamate is, in turn, finely controlled by changes mainly in the rate of synthesis, although changes in the rate of degradation of this compound may also be involved [11,12]. We have measured changes in the levels of carbamylphosphate synthetase and ornithine transcarbamylase and in the level of *N*-acetylglutamate in rats during fasting, and have studied the levels of the mitochondrial *N*-acetylglutamate synthetase and of the cytosolic *N*-acetylglutamate deacylase which are the key enzymes in *N*-acetylglutamate synthesis and degradation. The results of these studies are presented here.

2. MATERIALS AND METHODS

Carbamyl phosphate, *N*-acetylglutamate, *N*-acetyl methionine, acetyl CoA, and pyruvate kinase were from Sigma Chemical Co. (St Louis MO). Dowex 50-X8 and Dowex 1-X8 (200–400 mesh) were from Bio-Rad (Richmond CA). L-[^{14}C]Glutamic acid (30 mCi/mol) was supplied by Radiochemical Centre (Amersham). ATP and NADH were obtained from Boehringer and Soehne GmbH (Mannheim). All the other chemicals were reagent grade.

Male Wistar rats (180–220 g body wt) were used in the experiments and were fed a standard diet (crude protein content 18%) ad libitum. The fasting rats were placed in individual cages and deprived of food for 3 or 6 days, but were never without water. Urine was collected every 24 h by placing the animals in individual metabolic cages.

Rats were killed at the same time of day by decapitation and livers were rapidly removed and

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weighed. Portions (1 g) of liver were immediately homogenized in 19 ml ice-cold distilled water in an Ultraturrax homogenizer for three 10 s intervals at full speed, with cooling by immersion in an ice bath. Hepatocytes were isolated as in [13], and incubated as in [14]. *N*-Acetylglutamate was determined as in [15]. To calculate the recovery in the purification we used L-*N*-[^{14}C]acetylglutamate synthesized in our laboratory as in [16]. *N*-Acetylglutamate was determined by a specific assay based on the activation of carbamylphosphate synthetase [17] as modified in [18].

Carbamylphosphate synthetase and ornithine transcarbamylase were measured in whole liver homogenates [1]. *N*-Acetylglutamate synthetase was purified as in [19] up to the ammonium sulphate precipitation step, and the activity was measured according to them. *N*-Acetylglutamate deacylase was measured in the cytosolic fraction as in [20] using 15 mM *N*-acetylmethionine as substrate.

Arginine was determined as in [6]. Citrulline and urea were determined colorimetrically as in [21], methionine as in [22] and protein as in [23], with crystalline bovine serum albumin as standard.

3. RESULTS AND DISCUSSION

After 3 days of fasting there was an increase in the activity/g liver of carbamylphosphate synthetase and ornithine transcarbamylase; however, as shown, the activity per total liver changed little (fig.1). The level of *N*-acetylglutamate per g liver and per total liver increased after 3 and 6 days of fasting (table 1). *N*-Acetylglutamate synthetase and *N*-acetylglutamate deacylase activities per g liver and per total liver increased after 3 days of fasting; however, after 6 days of fasting both *N*-acetylglutamate synthetase and *N*-acetylglutamate deacylase activities per g liver and per total liver decreased (fig.2). These changes are associated with decreased body and liver weight and increased urinary urea excretion as well as increased urea synthesis by hepatocytes (table 1).

Since *N*-acetylglutamate appears to play an important role in determining the rate of urea synthesis under many circumstances [5–8], it was reasonable to find high levels of this activator during fasting, a situation in which ureagenesis and

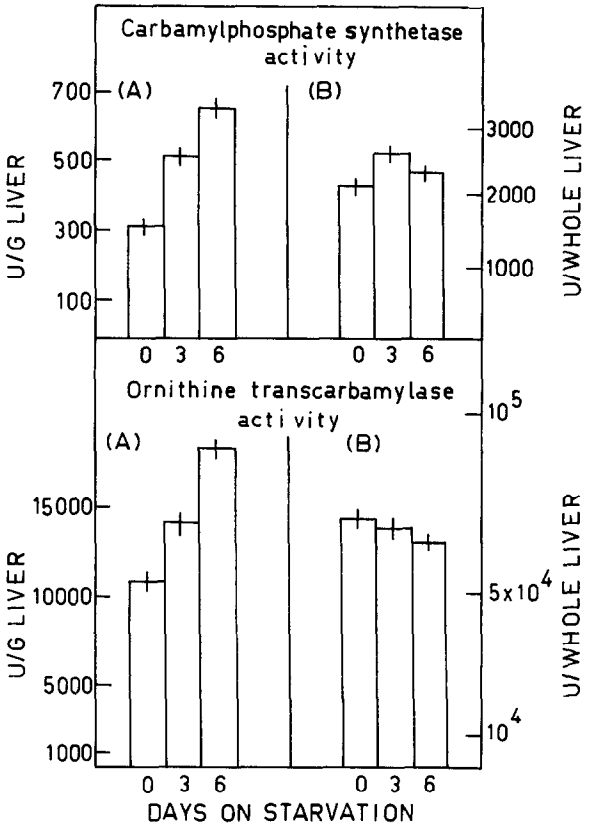

Fig.1. Effect of starvation on the enzymatic activity of carbamylphosphate synthetase and ornithine transcarbamylase per g liver (A) and per whole liver (B). U refers to 1 μmol citrulline/h for both enzymes. Each result is the average ± SD of 8 rats.

gluconeogenesis from amino acids are greatly increased [1].

High levels of *N*-acetylglutamate may result from increased synthesis or from decreased degradation of this compound [12]. The increased level of *N*-acetylglutamate synthetase found after a 3-day fast (fig.2) may be responsible for the increase in the amount of *N*-acetylglutamate. Other factors, and especially the increased levels of acetyl-CoA found during starvation [24], may also play a role in determining a more rapid synthesis of the compound.

Arginine levels did not change in fasting (not shown). Sensitivity to arginine, another key feature in the modulation of *N*-acetylglutamate synthetase activity, appears to be low or non-existent during fasting [25].

Table 1

Effect of starvation on body and liver weights, urea excretion, urea synthesized by hepatocytes and *N*-acetylglutamate level in rat liver

	Control	Starvation	
		3 days	6 days
Body wt (g)	201 ± 42	171 ± 35	143 ± 37
Liver wt (g)	6.5 ± 1.1	5.06 ± 0.97	3.55 ± 0.97
Urinary urea output	575 ± 32	861 ± 144	1431 ± 167
Urea synthesized	3.2 ± 0.9	4.6 ± 1.05	5.4 ± 0.8
N-Acetyl-glutamate	28.5 ± 4.5	38 ± 5	58 ± 8

Urinary urea output is expressed in mg/24 h; urea synthesized in isolated hepatocytes in μmol.min^{-1}.g wet wt^{-1}; *N*-acetylglutamate in nmol/g liver. Results represent the mean of 10 animals ± SE in each experimental group

After 6 days of fasting, the level of *N*-acetylglutamate synthetase decreased (fig.2). The restricted protein synthesis in extended fasting might account for this, especially if *N*-acetylglutamate synthetase has a short half-life. Despite the decreased synthetic activity in the longer period of fasting, *N*-acetylglutamate levels remained high (table 1), possibly the result of a slower degradation of this compound.

Two processes appear to be involved in *N*-acetylglutamate degradation, *N*-acetylglutamate degradation by cytosolic *N*-acetylglutamate deacylase [20] and *N*-acetylglutamate transport across the mitochondrial membrane [12]. *N*-Acetylglutamate deacylase activity is normally present in large amounts in the cytosol and thus *N*-acetylglutamate is degraded rapidly when it reaches this cell compartment [12]. We have measured the activity of *N*-acetylglutamate deacylase and have found it was decreased at 6 days of fasting (fig.2). It is possible that lowered *N*-acetylglutamate deacylase levels may lead to a longer turnover time for *N*-acetylglutamate and thus to higher levels of this activator. This may partly account for the increased levels of the activator found after fasting and especially after 6 days, when *N*-acetylglutamate synthetase was decreased (fig.2).

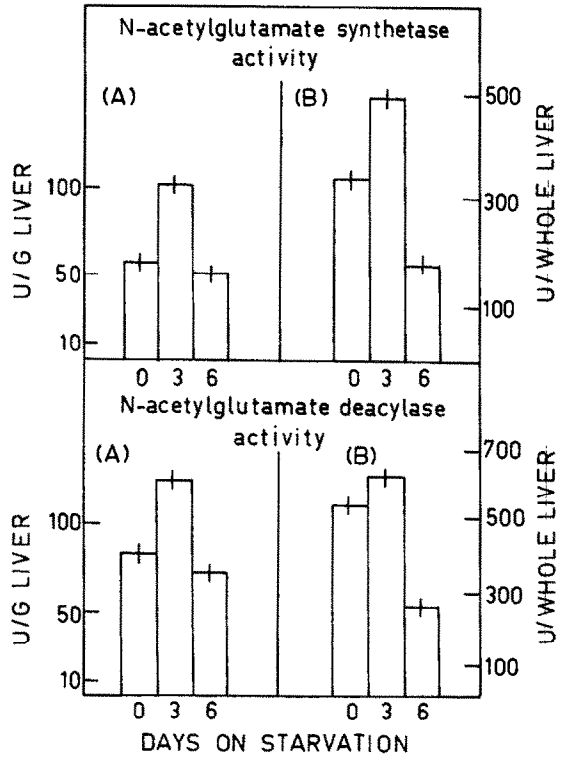

Fig.2. Effect of starvation on the enzymatic activity of *N*-acetylglutamate synthetase and *N*-acetylglutamate deacylase per g of liver (A) and per whole liver (B). U refers to 1 nmol *N*-acetylglutamate/h for *N*-acetylglutamate synthetase and to 1 μmol methionine/h for *N*-acetylglutamate deacylase. Each result is the average ± SD of 8 rats.

It has been suggested recently that transport determines the rate of *N*-acetylglutamate degradation, but the higher levels of *N*-acetylglutamate shown after 3 days fasting may be the result of the relative increase of *N*-acetylglutamate synthetase over the *N*-acetylglutamate deacylase activity.

In spite of the decrease in *N*-acetylglutamate synthetase activity after 6 days of fasting, the ratio of *N*-acetylglutamate synthetase/*N*-acetylglutamate deacylase activities is higher at 6 days than in the control group, which could explain, in part, the higher hepatic level of *N*-acetylglutamate found after 6 days of fasting, without ruling out the influence that *N*-acetylglutamate transport across the mitochondrial membrane may have.

We conclude that the increased urea excretion can be correlated with the increase in urea synthesis by isolated hepatocytes and the greater car-

bamylphosphate synthetase activity, which, in turn, is modulated by changes in the *N*-acetylglutamate levels, possibly resulting from increases in acetyl-CoA and decreased *N*-acetylglutamate deacylase activity. Moreover, in fasting there is an increase in glucagon, which, according to [7], increases per se mitochondrial *N*-acetylglutamate.

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