Arginine activation of *N*-acetylglutamate synthetase in mouse liver

Enhancement of the sensitivity in vivo by parenteral treatment with inhibitors of nucleic acid and protein synthesis

Susumu Kawamoto and Masamiti Tatibana*

Department of Biochemistry, Chiba University School of Medicine, Inohana, Chiba 280, Japan

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N-Acetyl-L-glutamate synthetase catalyzes the synthesis of *N*-acetyl-L-glutamate, an allosteric and essential activator of carbamoyl-phosphate synthetase I in the liver of ureotelic animals. The enzyme is activated specifically by arginine. We report here that the sensitivity of the synthetase to activation by arginine increases markedly after intraperitoneal administration to mice of inhibitors of nucleic acid and protein synthesis, including actinomycin D, aurintricarboxylic acid, cycloheximide, emetine and puromycin. The effects of cycloheximide were investigated in detail and an amino acid analysis was made of the homogenate of freeze-clamped livers of control or cycloheximide-treated mice.

Mitochondria *Urea synthesis* *Acetylglutamate synthetase* *Arginine* *Cycloheximide* *Carbamoyl-phosphate synthetase I*

1. INTRODUCTION

We have proposed [1–5] that *N*-acetyl-L-glutamate, a specific and obligatory allosteric activator of mitochondrial carbamoyl-phosphate synthetase I (EC 6.3.4.16) [6,7], participates in the control of urea biosynthesis in the liver of ureotelic animals, through the mediation of its hepatic level. The control mechanism has been supported by findings of several other research groups [8–13] (review [14]). We first obtained evidence for the presence of acetylglutamate synthetase (EC 2.3.1.1) [15], the enzyme which catalyzes the formation of acetylglutamate from glutamate and acetyl-CoA, in mitochondria of mammalian liver. The enzyme was partially purified from rat liver [16,17] and from human liver [18]. A unique property of this enzyme is the activation by arginine. In [19] the sensitivity of the synthetase to activation by arginine underwent marked changes after ingestion of food; these changes appeared to be due to a modification of the enzyme molecule itself [19].

We now report that the sensitivity of the synthetase to arginine activation increases markedly after intraperitoneal administration of inhibitors of nucleic acid and protein synthesis, irrespective of dietary conditions.

2. MATERIALS AND METHODS

Male dd/Y strain mice, 5–6 weeks old, 15–20 g body wt, were used throughout. Ammonium aurintricarboxylate, cycloheximide and emetine·HCl were obtained from Nakarai Chemicals (Kyoto). Actinomycin D and puromycin·2 HCl were from Makor Chemicals (Jerusalem) and American Cyanamid (New York), respectively.

* To whom correspondence should be addressed

Abbreviation: acetylglutamate, *N*-acetyl-L-glutamate

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Acetylglutamate synthetase in the sonicated mitochondria was measured, as in [19], except that the isolated mitochondria were suspended in 50 mM potassium phosphate buffer (pH 7.2) containing 0.5 mM dithiothreitol. Protein was determined as in [19].

Determination of amino acids in the liver was carried out as follows. Livers were freeze-clamped, powdered and homogenized with 5 vol. 1 M perchloric acid in a Polytron homogenizer (Kinematica GmbH, Luzern). The extract and the washings of the residue were combined and then analyzed using a Hitachi amino acid analyzer, model 835 (Hitachi, Tokyo).

3. RESULTS

Various inhibitors of nucleic acid and protein synthesis were given intraperitoneally to fasted mice and the activity and arginine sensitivity of acetylglutamate synthetase were examined after 8 h (table 1). When mice were given 0.15 M NaCl (control mice), the activities both in the presence and absence of arginine (1 mM) were low, and the extent of arginine activation was 1.6. On the contrary, when mice were given inhibitors of protein synthesis, including aurintricarboxylate, cycloheximide, emetine and puromycin, the activity in the absence of arginine increased several times, whereas the activity in the presence of arginine increased to a remarkably greater extent (>15-fold). As a consequence, the extent of arginine activation increased 6–9-fold. When mice were treated with actinomycin D, an inhibitor of ribonucleic acid synthesis, the extent of arginine activation remained as low as 3.6-fold.

Of the 5 inhibitors of nucleic acid and protein synthesis, effects of cycloheximide were in-

Table 1

Changes in activity of acetylglutamate synthetase after treatment of mice with inhibitors of nucleic acid and protein synthesis

Treatment	Acetylglutamate synthetase activity (nmol.h^{-1}.mg protein^{-1})		Activation by arginine (-fold)
	−Arg	+Arg	
Control	0.13	0.21	1.6
Actinomycin D	0.31	1.11	3.6
Aurintricarboxylate	0.51	3.38	6.6
Cycloheximide	0.33	2.95	8.9
Emetine	0.71	4.47	6.3
Puromycin	0.37	3.44	9.3

Six groups of 3 mice each were fed the usual laboratory chow for 3 h (from 9.00 pm–12.00 pm) for 3 days and were then fasted for 33 h and given actinomycin D, cycloheximide or emetine (1, 50 or 5 μg/g body wt, respectively) after 0, 3 and 6 h, aurintricarboxylate or puromycin (3 or 75 μg/g body wt, respectively) at each hour. Control mice were given only 0.15 M NaCl after 0, 3 and 6 h. All mice were killed after 8 h and mitochondria were isolated from the pooled 3 livers, suspended and sonicated. The enzyme activity was measured in the presence and absence of 1 mM L-arginine

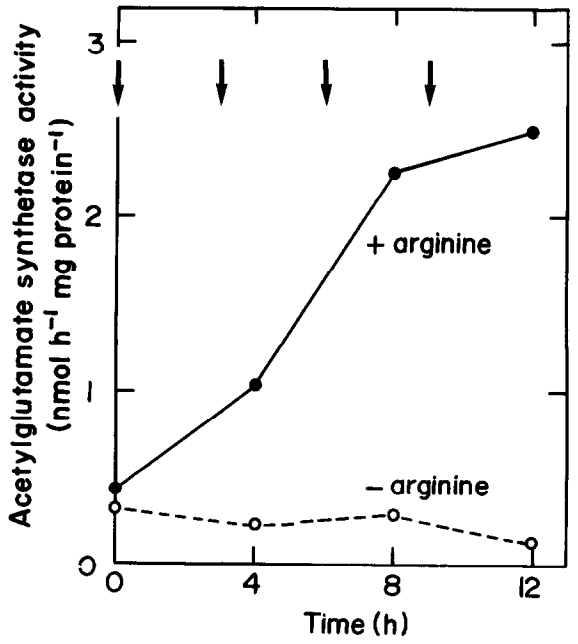

Fig.1. Changes in acetylglutamate synthetase activity during the course of cycloheximide treatment of mice. A total of 12 mice were fed and fasted as in table 1. On day 5, 3 animals were killed at 0-time. The other animals were given cycloheximide (50 μg/g body wt) at 3-h intervals as indicated by the arrows. Three mice were killed every 4 h. The enzyme activity in the sonicated liver mitochondria was measured in the presence (●) and absence (○) of 1 mM L-arginine as in table 1.

vestigated in greater detail. When the agent (50 μg/g body wt) was repeatedly given to fasted mice at 3-h intervals, the activity in the absence of arginine remained at similar levels throughout the entire period of 12 h (fig.1). The activity in the presence of arginine increased remarkably with time, and there was a marked increase in the extent of arginine activation. Dose response of the synthetase activity to cycloheximide is illustrated in fig.2. Mice were given various doses (0, 10, 20, 50, 100 or 200 μg/g body wt) of cycloheximide 3-times at 3-h intervals and the hepatic enzyme activity was examined after 8 h. The activity assayed in the absence of arginine did not significantly change, while the activity assayed in the presence of arginine increased with increasing doses up to about 20 μg/g body wt for a single injection. The maximal arginine activation ratio attained by treatment with cycloheximide was 8–11-fold when the dose was over 20 μg/g body wt. When liver slices were incubated with [^{35}S]methionine, the incorporation of radioactivity into the trichloroacetic acid-insoluble fraction of 7500 × *g* precipitate or supernatant of liver homogenate from the antibiotic-treated mice was only 7–9% of that from control mice (not shown). The hepatic protein synthesis of cycloheximide-treated mice appears to be strongly repressed.

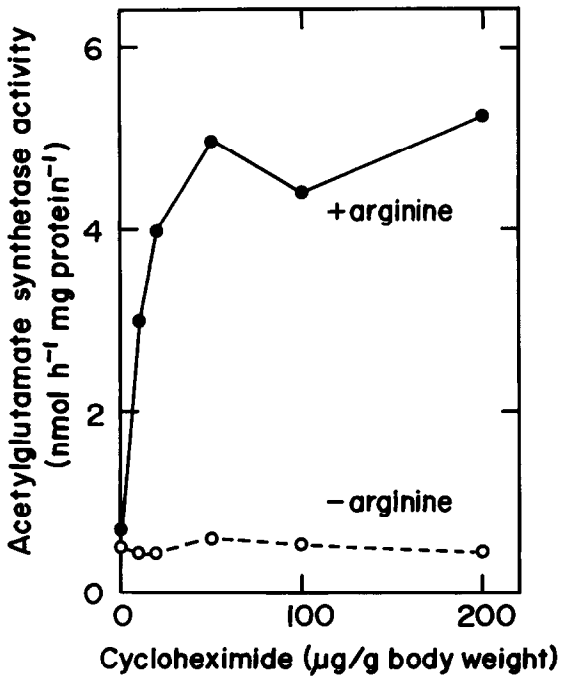

Fig.2. Dose response of acetylglutamate synthetase activity to cycloheximide. Six groups of 3 animals were fed and fasted as in table 1. On day 5, cycloheximide (0, 10, 20, 50, 100 or 200 μg/g body wt) was given to mice after 0, 3 and 6 h. All mice were killed after 8 h and the enzyme activity in the sonicated liver mitochondria was measured in the presence (●) and absence (○) of 1 mM L-arginine as in table 1.

Content of each amino acid in the homogenate of freeze-clamped livers of control or cycloheximide-treated mice was measured (table 2). The hepatic levels of some amino acids, including citrulline, valine, isoleucine, leucine, tyrosine, lysine and arginine, in the treated mice were higher than in the controls, while the levels of some amino acids, including glutamic acid, glycine, phenylalanine and histidine, were lower. The total amount of amino acids in the livers of the treated mice was about 110% of that of the control mice.

4. DISCUSSION

Factors known to be involved in the control of acetylglutamate synthetase activity are the intracellular levels of glutamate and acetyl-CoA [3], substrates, the concentrations of competitive inhibitors, such as propionyl-CoA [20], the hepatic content of arginine [3], a specific activator of the synthetase and the changes in sensitivity of the synthetase to arginine activation [19]. It was also found [19] that cycloheximide injection, which inhibited the total protein synthesis in the mouse liver by 90%, did not inhibit the postprandial increase in arginine activation of the synthetase, thus indicating that the protein synthesis is not required for the change; actually cycloheximide stimulated the increase to a significant extent. Here, we demonstrated that arginine sensitivity of the synthetase increased markedly after injection of inhibitors of protein synthesis, including aurintricarboxylate, cycloheximide, emetine and puromycin, irrespective of dietary conditions (table 1). With actinomycin D, an inhibitor of DNA-dependent RNA synthesis, there was a smaller increase in arginine sensitivity, probably due to delay in the cessation of protein synthesis. Regardless of the same or different mechanisms by

Table 2

Effects of cycloheximide on hepatic amino acid levels in mice

Amino acid	Control mice	Treated mice	
	(μmol·g liver^{-1})	(μmol·g liver^{-1})	(% control)
Taurine	15.93 ± 1.38	18.06 ± 0.77	113
Aspartic acid	2.54 ± 0.19	2.13 ± 0.36	84
Threonine	0.41 ± 0.08	0.57 ± 0.05	139
Serine	0.52 ± 0.15	0.69 ± 0.06	133
Glutamic acid	1.61 ± 0.44	0.89 ± 0.14	55
Glutamine	2.77 ± 0.65	3.91 ± 0.24	141
Glycine	1.51 ± 0.19	0.98 ± 0.08	65
Alanine	0.58 ± 0.53	0.42 ± 0.20	72
Citrulline	0.34 ± 0.29	0.88 ± 0.44	259
Valine	0.40 ± 0.14	0.64 ± 0.05	160
Methionine	0.09 ± 0.02	0.10 ± 0.00	111
Isoleucine	0.20 ± 0.05	0.30 ± 0.04	150
Leucine	0.32 ± 0.06	0.48 ± 0.04	150
Tyrosine	0.04 ± 0.03	0.06 ± 0.01	150
Phenylalanine	0.09 ± 0.01	0.05 ± 0.01	56
Ornithine	0.85 ± 0.12	0.69 ± 0.07	81
Lysine	0.66 ± 0.26	1.33 ± 0.33	202
Histidine	0.48 ± 0.08	0.30 ± 0.09	63
Arginine	0.03 ± 0.04	0.13 ± 0.03	433
Total	29.35 ± 0.95	32.56 ± 0.16	111

A total of 7 mice were fed and fasted as in table 1. On day 5, 4 mice were given cycloheximide (50 μg/g body wt) after 0, 3 and 6 h. Control animals were given 0.15 M NaCl. All mice were killed after 8 h and analysis of amino acids in the liver was done as described in section 2. Values represent the mean value ± SD (3 control, and 4 treated animals)

which the inhibitors bring about the increase in arginine sensitivity of the synthetase and by which diet causes similar changes, the increases induced by these inhibitors will aid in elucidating postprandial changes. Inhibition of synthesis of protein factor(s), with a short half-life, may produce increases in arginine sensitivity of the synthetase, following administration of cycloheximide to mice.

Table 2 shows that the hepatic level of total amino acids of cycloheximide-treated mice increased to some extent, in comparison to levels seen in control mice, and that the level of some amino acids increased significantly in the treated mice. How these changes relate to increases in arginine sensitivity is the subject of ongoing investigation in our laboratory.

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