ACTIVITY OF THE ENZYMES OF CREATINE BIOSYNTHESIS IN THE PANCREAS OF RATS POISONED WITH ETHIONINE

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Intraperitoneal injection of DL-ethionine in a dose of 1 g/kg into rats completely suppresses the activity of guanidine acetate-methyl transferase (GAMT; 2.1.1.2), an enzyme of the second stage of creatine synthesis (3-48 h after poisoning). The level of activity of glycine-amidinotransferase (GLAT; 2.1.4.1), the enzyme of the second stage of creatine synthesis, rises sharply 3 h after poisoning but then gradually falls. The increase in GLAT activity 3 h after poisoning of the rats with ethionine is accompanied by a 6-7-fold decrease in the creatine concentration in the pancreas. This stimulation of enzyme activity is suppressed by actinomycin and cycloheximide, inhibitors of protein synthesis. The creatine concentration in in the pancreas, meanwhile, is increased.

The biochemical mechanism of damage to the pancreas by ethionine, unlike that in the liver, is not significantly attributable to synthesis of an abnormal protein containing ethionine instead of methionin [8, 10]. A more important factor [8] is the formation of S-adenosylethionine (SAET) and its accumulation in the pancreas.

Since creatine synthesis is one of the specific functions of the pancreas [15], in the investigation described below the effect of ethionine poisoning was studied on the enzyme system of creatine biosynthesis. The structural similarity between SAET and the natural substance S-adenosylmethionine (SAME), which participates in creatine biosynthesis, suggested that SAET possibly acts as an antimetabolite on the methylation of guanidine-acetic acid.

EXPERIMENTAL METHOD

Male Wistar rats weighing 200-250 g, kept on a standard diet, were used. The animals of the experimental and control groups were kept without food and water for 12 h before the beginning of the experiment. DL-ethionine was injected intraperitoneally in 2% aqueous solution in a dose of 1 g/kg body weight. Control animals received 0.9% NaCl solution. The animals were sacrificed by decapitation 3, 6, 12, 24, and 48 h after injection of the poison. In the series of experiments in which rats were poisoned with ethionine while protein synthesis was blocked by actinomycin or cycloheximide, the animals were sacrificed 3 h after the injection of ethionine. Glycine-amidinotransferase (GLAT) was determined by the method of Van Pilsum et al. [14] in the writer's modification [2]. Activity was expressed in micromoles guanidineacetic acid (GAA) formed during incubation for 1 h at 37°C per gram dry pancreatic tissue.

Guanidine-acetate-methyl transferase (GAMT) was determined by the method of Cantoni and Vignos [4] with slight modifications.

GAMT activity was expressed in micromoles creatine (creatinine) synthesized during incubation for 4 h per gram dry pancreatic tissue. To determine the dry residue, a weighed sample of tissue was dried at 105°C to constant weight. Creatine preformed in the pancreatic tissue was determined by Borsook's method [3] using Lloyd's reagent.

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TABLE 1. Effect of Poisoning Rats with Ethionine on GLAT and GAMT Activity in Pancreas and Change in Activity of These Enzymes Produced by Methionine, Actinomycin D, and Cycloheximide (M ± m)

Series of experiments	Agent injected	Time (in h)	GLAT activity (in μ moles/g per hour at 37°C)	GAMT activity (in μ moles/g per 4 h)	Creatinine concentration (in μ moles/g)
I	0.9% NaCl solution (control)		7,58±0,77 (6)	3,24±0,33 (4)	—
		3	22,26±1,70 (5)	0 (4)	—
		6	7,01±0,28 (5)	0 (4)	—
		12	4,43±1,86 (6)	0 (4)	—
		24	3,98±0,25 (5)	0 (5)	—
		48	0,75±0,12 (5)	0 (5)	—
II	DL-ethionine + L-methionine	6	20,51±0,88 (5)	0	—
		12	12,30±0,71 (5)	0,67±0,09 (4)	—
		24	5,57±0,55 (6)	1,54±0,16 (5)	—
III	0.9% NaCl solution (control)	3	8,52±1,13 (5)	—	5,72±0,21 (5)
	DL-ethionine	3	25,02±2,50 (4)	—	0,87±0,09 (4)
	DL-ethionine + actinomycin (30 μg/100 g)	3			
	DL-ethionine + cycloheximide (100 μg/100g)	3	12,75±0,56 (5)	—	3,02±0,2 (4)
		3	12,66±0,54 (5)	—	4,10±0,14 (5)

Note. Control rats (experiments of series I) received injection of 12 ml 0.9% NaCl solution 12 h before sacrifice. DL-ethionine was injected intraperitoneally in a dose of 100 mg/100 g body weight. An aqueous solution of methionine was injected intraperitoneally in a dose of 100 mg/100 g body weight. The antibiotics were dissolved in 0.9% NaCl solution and injected intraperitoneally 1 h before the injection of ethionine. Number of animals given in parentheses.

EXPERIMENTAL RESULTS

In the experiments of series I after injection of 0.9% NaCl solution into the rats the GLAT (transamidinase) activity was 7.58 ± 0.77 μ mole/g per hour; the GAMT activity was 3.24 ± 0.33 μ mole/g per 4 h.

As the results in Table 1 show, GLAT activity 3 h after poisoning of the rats with ethionine was increased by 3 times, after which it gradually fell to its initial level which was reached 6 h after poisoning.

GAMT activity during this period was completely suppressed. Injection of ethionine in a dose of 100 mg/100 g body weight into the rats thus blocked the enzymic transport of methyl groups from SAME to GAA (3-48 h). SAET probably inhibits the enzymic transmethylation of guanidine-acetic acid by S-adenosylmethionine by a competitive mechanism not only in the liver [12, 13], but also in the pancreas. GLAT activity fell gradually during this period, and 48 h after poisoning with ethionine it was 0.75 ± 0.12 μ mole/g per hour at 37°C.

In rats receiving ethionine together with an equivalent dose of L-methionine (100 mg/100 g body weight; experiments of series II) GAA synthesis from arginine and glycine under the influence of pancreatic homogenates was sharply increased after 6 h (20.51 ± 0.88 μ moles/g per hour), after which it decreased and after 12-14 h it was actually below its initial level (5.57 ± 0.55 μ moles/g per hour). Creatine synthesis from SAME and GAA increased gradually during this period. This probably occurred because the SAME, which was formed in the pancreas after injection of methionine, began to be utilized in the various transmethylation reactions [13], including in creatine synthesis.

Since, however, it has been shown that the ethyl group of SAET can be transported to different intracellular acceptors [11, 12] with the formation of the corresponding ethyl derivatives, a certain quantity of the ethyl analogue of creatine is presumably formed in the pancreas as a result of the similar interaction between SAET and GAA. The ethylation of arginine, which is related to creatine, possibly may occur also [6].

In rats poisoned with ethionine the natural metabolite creatine, which is a repressor of GLAT [16], is presumably replaced by its ethyl analogue. As a result, the creatine concentration in the pancreas must be reduced, and with it the pancreas must lose its ability to control GLAT with creatine by the negative feedback principle.

The results given in Table 1 (experiments of series II) show that in rats receiving injections of 0.9% NaCl the GLAT activity was 8.52 ± 1.13 μmoles/g per hour, whereas 3 h after injection of ethionine the level of GLAT activity was increased threefold (25.02 ± 2.50 μmoles/g per hour). Simultaneously with this the creatine concentration per gram dry weight of pancreas was reduced by 6-7 times compared with the control (0.87 ± 0.09 μmoles/g; normal 5.72 ± 0.21 μmoles/g). As a result of this "unblocking" of the gene, the increase in the level of pancreatic GLAT observed 3 h after poisoning with ethionine (derepression) evidently took place.

To obtain direct information on the nature of derepression of metabolic control of GLAT, actinomycin D, which blocks DNA-dependent RNA synthesis [7], was used. GLAT activity in the pancreas, measured 3 h after administration of ethionine to rats in which RNA synthesis was blocked with actinomycin, was 12.75 ± 0.56 μmoles/g per hour, and the creatine level was 3.02 ± 0.2 μmoles/g. An almost identical result was obtained in the experiment in which GLAT derepression was abolished by cycloheximide (an inhibitor of protein [5] and DNA [9] synthesis) (Table 1). However, the dose of the antibiotic required to obtain the stipulated effect was 3 times greater than the dose of actinomycin D, namely 100 μg/100 g body weight.

The results of these experiments thus show that stimulation of transamidinase in the rat pancreas 3 h after ethionine poisoning (derepression) is largely prevented in the presence of antibiotics (actinomycin D and cycloheximide) and it evidently takes place at the level of transcription of the template function of DNA. The enzyme activity remains high even after protein synthesis is blocked by ethionine in the presence of actinomycin (12.75 ± 0.56 μmoles/g per hour). This means that the mRNA which codes the synthesis of this enzyme is not split up [1]. The reason for the high GLAT activity in the pancreas of the rats during the 3-6 h after ethionine poisoning is that either the polyribosomal mRNA coding GLAT synthesis remains metabolically stable, or a reserve of mRNA specific for the enzyme exists in the acinar cell, probably in the nucleus.

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