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INDUCTION OF RAT LIVER GLYCINE METHYLTRANSFERASE BY HIGH METHIONINE DIET

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Summary: Dietary experiments were carried out to evaluate the physiological role of glycine methyltransferase. When rats received a 18% casein diet containing excess methionine, the activity of the enzyme in liver extracts increased with increasing methionine content in the diet. Adenosylmethionine synthetase and adenosylhomocysteinase activities were also elevated, while guanidoacetate methyltransferase activity showed no significant change. The glycine methyltransferase activity reached a maximal level after 4-6 days on the 3% methionine diet. Immunological titration showed that the increase in activity was associated with the increase in amount of the enzyme.

Glycine methyltransferase (S-adenosyl-L-methionine: glycine methyltransferase, EC 2.1.1.20) catalyzes the S-adenosylmethionine-dependent methylation of glycine to yield sarcosine. The enzyme from rat liver is a simple protein consisting of four identical subunits with M_r = 31500, and exhibits a positive kinetic cooperativity toward S-adenosylmethionine (1). The activity of the enzyme appears to be the highest of methyltransferases in adult liver (1,2), but it is virtually absent in rat hepatoma cells (3,4) and very low in the fetal or regenerating liver (4). Although it is suggested that the enzyme is involved in the oxidation of methyl carbon of methionine (5), or in the regulation of relative levels of S-adenosylmethionine and S-adenosylhomocysteine (2), its physiological role is not well understood. As a first step to assess the physiological role of the methyltransferase, we have studied the effect of high methionine diet on the hepatic glycine methyltransferase activity. In the transsulfuration pathway of methionine degradation, S-adenosylmethionine is converted to S-adenosylhomocysteine by donating its methyl group to various acceptors. If glycine methyltransferase plays a major part in this transformation, it would be expected that the enzyme activity increases by feeding excess methionine. This communication reports that the activity of glycine methyltransferase as well as that of adenosylmethionine synthetase (EC 2.5.1.6)

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increase in the liver when rats are fed on a high methionine diet, and the rise of glycine methyltransferase activity is due to the enzyme induction.

EXPERIMENTAL PROCEDURES

Animals and Diets. Male Wistar rats weighing approximately 100 g were obtained from Hokuriku Laboware Co. (Toyama). They were housed in wire-bottom cages in a room of a 12-h light-dark cycle. The basal diet contained, in g per kg, casein, 180; cornstarch, 579; sucrose, 150; soybean oil, 20; salt mix (6), 40; vitamin mix (6), 10; cellulose powder, 20; choline chloride, 1. Components of the diet were products of Oriental Yeast Co. (Tokyo). When the diet was supplemented with L-methionine or glycine, the amount of cornstarch was reduced by the amount of the additions.

Chemicals. S-Adenosyl-L-methionine, S-adenosyl-L-homocysteine, and adenosine deaminase (calf intestinal mucosa) (EC 3.5.4.4) were purchased from Sigma. S-Adenosyl-[^{14}C-methyl]-L-methionine (60 mCi/mmol) was obtained from Amersham, and [^{14}C-methyl]-L-methionine (13.8 mCi/mmol) from New England Nuclear. Other chemicals were obtained locally from commercial sources, and were used without further purification.

Preparation of Liver Extracts. Rats were killed by decapitation and their livers were quickly removed and chilled in ice. The livers were homogenized in a blender for 1 min (18000 rpm) with 5 volumes of cold 10 mM potassium phosphate, pH 7.2, containing 1 mM EDTA. The homogenate was centrifuged for 30 min at 10000 X g and the supernatant was used for enzyme assays. For the measurement of adenosylmethionine synthetase activity, the extract was diluted further with 2 volumes of 10 mM Tris/HCl, pH 7.4, containing 20% glycerol, 10 mM $MgCl_2$, 1 mM dithiothreitol and 150 mM KCl.

Enzyme Assays. Glycine methyltransferase activity was determined as described previously (1). Adenosylmethionine synthetase was assayed by the method of Liau et al. (7). Adenosylhomocysteinase (EC 3.3.1.1) activity was measured in the direction of S-adenosylhomocysteine hydrolysis (8). Guanidoacetate methyltransferase (EC 2.1.1.2) was assayed according to Im et al. (9), except that the thiol reagent was omitted from the reaction mixture. In a crude liver extract, the enzyme required no thiol for activity (Ogawa and Fujioka, unpublished observation). Under the assay conditions, the activity of each enzyme was linear with respect to time and enzyme concentration. One unit of enzyme activity was defined as the amount of enzyme forming 1 nmol of product/min at 37 °C.

Other Methods. Protein was determined by the micro-biuret method (10) using bovine serum albumin as a standard. The antiserum against the purified rat liver glycine methyltransferase was prepared in rabbits as described previously (1). The mean and standard error was calculated for each treated group and for control group. The significance of differences of the means was calculated by Student's t test.

RESULTS

Effect of Methionine Diets on Activities of Glycine Methyltransferase, Adenosylmethionine Synthetase, Adenosylhomocysteinase, and Guanidoacetate Methyltransferase. After 3 to 5 days on the basal diet, rats were fed the basal diets supplemented with various amounts of L-methionine and/or 3% glycine for 4 days. As seen in Table I, rats maintained on the 0.5% methionine diet showed the greatest weight gain. Increase of the methionine content to 1.5%

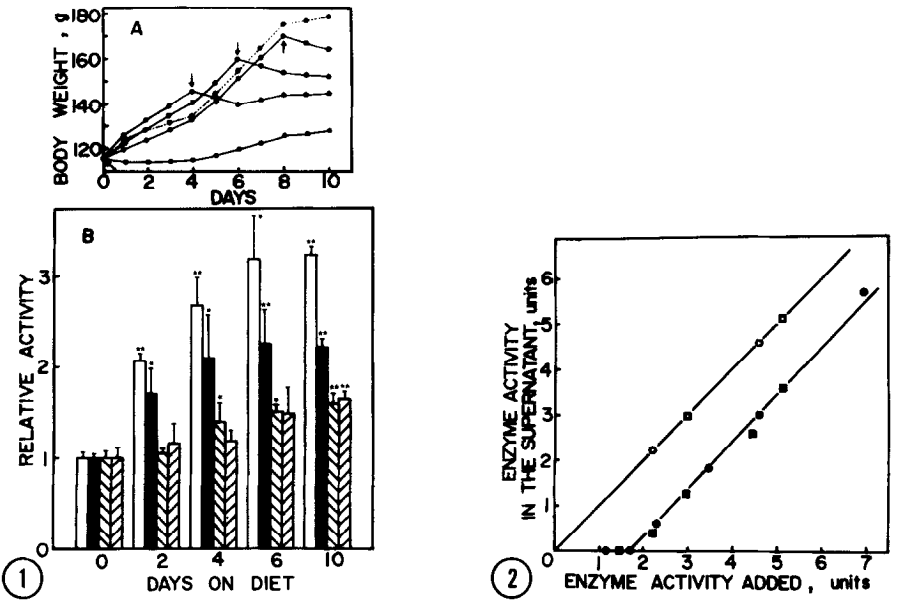

Fig. 1. Growth curve of rats on the 3% methionine diet (A), and enzyme activities as a function of time (B). A, the diet was changed from the basal to the 3% methionine diet at times indicated by arrows. Rats received the 3% methionine diet thereafter. Body weights represent the averages of 4 rats. B, enzyme activities are expressed as relative activities obtained by comparison with those of the control group. The specific activities in the control were 0.35, 1.01, 5.58, and 0.19 units/mg protein for adenosylmethionine synthetase (□), glycine methyltransferase (■), adenosylhomocysteinase (▧), and guanidoacetate methyltransferase (▨), respectively. *, $p < 0.01$; **, $p < 0.001$.

Fig. 2. Immunological titration of liver extracts with antiserum against glycine methyltransferase. Equal volumes of liver extracts from 4 rats fed the basal diet and the 3% methionine diet for 4 days were each combined. Varying amounts of glycine methyltransferase activity contained in extracts were added to 20 µl (1.8 mg protein) of anti-glycine methyltransferase antiserum and to the same volume of an unimmunized rabbit serum. After incubation for 1 h at room temperature and then at 4 °C overnight, the supernatants were obtained by centrifugation and assayed. □, control liver extract + unimmunized serum; ○, methionine-diet liver extract + unimmunized serum; ■, control liver extract + antiserum; ●, methionine-diet liver extract + antiserum.

resulted in a depression of growth. As noted earlier (11), high concentrations of methionine in the diet were toxic, and rats given the 3% methionine diet lost their body weights during the 4-day period. When examined on the fifth day, the hepatic activity of glycine methyltransferase was found to increase with increasing methionine content. A similar rise of activity was observed with adenosylmethionine synthetase. Adenosylhomocysteinase activity showed a much less pronounced increase over the control. In contrast, guanidoacetate methyltransferase, which has a rather high activity in liver extract among methyltransferases, was refractory to the methionine treatment. Although sim-

ultaneous addition of glycine (3%) to the 3% methionine diet alleviated the methionine toxicity as seen by the body weight change, it did not prevent the rise of activities of the three enzymes.

Time Course of Change in Enzyme Activities. When the diet was changed from the basal diet to the one containing 3% methionine, the growth of rats ceased immediately (Fig. 1A). Rats maintained on the high methionine diet for a prolonged period gradually gained their body weights apparently due to adaptation to the diet. On the 3% methionine diet, a parallel increase with time in activities of glycine methyltransferase and adenosylmethionine synthetase was observed (Fig. 1B). These activities reached their maximal levels in 4 to 6 days. Adenosylhomocysteinase and guanidoacetate methyltransferase activities were seen elevated only after 4 and 6 days of the treatment, respectively.

Immunotitration of Glycine Methyltransferase. To test whether the increase in glycine methyltransferase activity is due to the increase in amount of the enzyme or to the change in its catalytic activity, immunotitration was carried out with the rabbit antiserum raised against the enzyme. As shown in Fig. 2, the equivalence point was not different with the glycine methyltransferase activity derived from the livers of rats fed a high methionine diet and that from the control livers. The result indicates that a 2.7-fold difference in specific activity between the two groups is brought about by the proportionate change in the amount of immunoprecipitable enzyme protein.

DISCUSSION

L-Methionine is the most toxic of amino acids in animals when given in excess over other amino acids (11). The toxic effects of methionine can be alleviated by simultaneously feeding glycine or serine, glycine being more effective than serine (12). Since serine is readily converted to glycine, this observation suggests that glycine is involved in the metabolism of methionine.

The results presented in this report demonstrate that glycine methyltransferase activity in the liver is increased by feeding rats on a high methionine diet. The increase in activity is the result of enzyme induction. The induc-

Table I. Effect of Dietary Methionine and/or Glycine on Enzyme Activities

	Weight gain	Adenosylmethionine synthetase	Glycine methyltransferase	Adenosylhomocysteinase	Guanidoacetate methyltransferase
	g	units/mg	units/mg	units/mg	units/mg
Control	22.5 ± 3.3	0.38 ± 0.22	0.79 ± 0.04	4.95 ± 0.25	0.21 ± 0.02
0.5% Met	27.8 ± 1.3	0.42 ± 0.05	0.83 ± 0.07	5.40 ± 0.51	0.20 ± 0.01
1.5% Met	12.0 ± 2.6	0.56 ± 0.04**	1.27 ± 0.20*	5.76 ± 0.83	0.21 ± 0.06
3.0% Met	-2.8 ± 2.5	0.95 + 0.07**	2.11 ± 0.30**	6.66 ± 0.48*	0.23 ± 0.01
3.0% Gly	20.0 ± 0.8	0.41 ± 0.04	0.71 ± 0.05	4.80 ± 0.14	0.20 ± 0.03
3.0% Met + 3.0% Gly	6.0 ± 1.2	0.76 ± 0.06**	1.98 ± 0.22**	7.47 ± 0.71**	0.23 ± 0.03

After feeding the basal diet for 3-5 days, rats received the diet indicated for 4 days. Enzyme activities were determined as described under "Experimental Procedures." Values represent the means ± S. E. of 4 rats. *, $p < 0.01$; **, $p < 0.001$. Abbreviations: Met, L-methionine; Gly, glycine.

tion of the methyltransferase is accompanied by a parallel increase in activity of adenosylmethionine synthetase, the first enzyme of the transsulfuration pathway of methionine metabolism. The immediate and apparently coordinate response of these activities to methionine feeding and the fact that the activity of glycine methyltransferase is the highest of known methyltransferases in liver extracts (1,2) suggest that this enzyme plays an important role in methionine metabolism by the transsulfuration pathway. Guanidoacetate methyltransferase, which is less active than glycine methyltransferase, is rather resistant to the methionine treatment (Table I and Fig. 1B).

In spite of the induction of glycine methyltransferase, the growth of rats fed on a high methionine diet is greatly depressed. This may be explained by a marked reduction of the hepatic pool size of serine and glycine after methionine feeding. Sanchez and Swendseid (13) reported that the contents of serine and glycine in the livers of rats fed a 4% methionine diet for 3 days fell to about 17 and 33%, respectively, of the control. Benevenga and his associates have shown that methionine is also degraded *via* transamination pathway. Transamination of methionine yields α-keto-γ-methiolbutyrate, which decarboxylates to 3-methylthiopropionate (14). Since the administration of 3-methylthiopropionate produces the same toxic effects as methionine (15), it may be suggested that intermediate(s) of the transamination pathway is responsible

for the toxicity. Reduction of the cellular pool of glycine would divert the metabolism of methionine to the transamination pathway even when glycine methyltransferase activity is increased.

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