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REGULATION OF HEPATIC BETAINE-HOMOCYSTEINE METHYLTRANSFERASE BY DIETARY METHIONINE

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SUMMARY: The hepatic activity of betaine-homocysteine methyltransferase is a complex function of the content of methionine in the diet. Enzyme levels are lower in the livers of rats fed a 0.3% methionine diet than in livers of animals maintained on either methionine-free or excessive-methionine (1.0%) rations. The finding that activities are increased at both extremes of the spectrum of dietary methionine intake suggests the possibility that the betaine-homocysteine methyltransferase reaction may function both to maintain tissue concentrations of methionine when intake of this amino acid is limited and to remove homocysteine when methionine intake is excessive.

INTRODUCTION

Betaine-homocysteine methyltransferase (EC 2.1.1.5) catalyzes a reaction essential for the catabolism of choline in mammalian tissues. In addition this reaction may be significant in the regulation of methionine metabolism. Together with cystathionine-β-synthase (EC 4.2.1.22) and 5-methyltetrahydrofolate homocysteine methyltranferase (EC 2.1.1.13), the betaine methyltransferase appears to constitute a regulatory locus (1). Homocysteine, derived from methionine, may be conserved by remethylation or may be committed irreversibly to the transsulfuration pathway.

The relative contributions of the two homocysteine methylases to the resynthesis of methionine remains to be defined. In a previous study (2) we found that the level of betaine-homocysteine methyltransferase in rat liver was increased in animals fed a high-protein diet and was induced by the injection of methionine. In contrast, both the methionine injection and the high-protein feeding reduced the hepatic content of 5-methyltetrahydrofolate homocysteine methyltransferase. We concluded that the folate enzyme was more likely to be involved in the maintenance

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of tissue levels of methionine during periods when the dietary intake of this amino acid was restricted. Betaine-homocysteine methyltransferase appeared to function primarily as a means for choline catabolism and to reduce the tissue content of homocysteine.

In a more recent study, however, we noted that the hepatic concentration of methionine fell significantly in rats fed a purified choline-free diet which contained only 0.3% methionine (3). Since there was no reason to postulate any impairment of the methyltetrahydrofolate reaction due to either diminished availability of homocysteine or reduction in the level of enzyme protein, we considered the possibility that betaine homocysteine methyltransferase may be essential for the conservation of methionine under certain nutritional conditions.

MATERIALS AND METHODS

Animals and Diets. We used male, Sprague-Dawley rats weighing 150-250 grams. The purified amino acid diet of Rogers and Newberne was the basis for our preparations (4). The latter were isocaloric and the contents of vitamins, lipids, salts and cystine (0.5%) were constant. In individual experiments we varied the concentrations of methionine, choline and non-sulfur containing amino acids.

Assays. We have described our methods for the determinations of betaine and choline in rat liver (3,5). The assay for betaine-homocysteine methyltransferase was a modification of the published procedure (6). The concentration of betaine was increased to 2 mM. We defined one unit of enzyme activity as the amount which catalyzes the methylation of 1 nmole of homocysteine in 60 min. Protein was determined by the Bio-Rad Method (Bio-Rad Laboratories, Richmond, CA.).

Expression of Results. Data is presented as the mean and the standard deviation from the mean for each group of determinations. We used the t-test for unpaired samples when we tested the significance of differences between groups.

RESULTS AND DISCUSSION

Effect of Dietary Amino Acids. Table I summarizes the results of experiments in which we varied the dietary content of amino acids other than methionine and cystine which were maintained at 0.3% and 0.5% respectively. Both diets contained 0.2% choline. As shown in the table, ingestion of the 5% amino acid-diet resulted in a significant increase in the hepatic content of betaine but no change in the level of choline. The concentration of soluble protein declined from 108 to

TABLE I
EFFECT OF DIETARY AMINO ACID CONTENT ON BETAINE AND BETAINE-HOMOCYSTEINE METHYLASE IN RAT LIVER

Diet	Choline nmol/g	Betaine µmol/g	Betaine Methyltransferase U/mg prot	U/g liver	U/liver/gBW
5% AA	156±39	3.1±1.0	39.6±6.3	3480±557	170±37
22% AA	128±39	1.1±1.3	40.9±9.0	4254±745	212±43
P		<.01		<.05	<.02

Each group consisted of ten rats which were fed the designated diet for five days. The diets were isocaloric and both contained 0.3% methionine and 0.2% choline.

89 mg/g ($P<0.001$). Consequently the decrease in betaine-homocysteine methyltransferase was not specific. While the U/g liver and total hepatic activity per g body weight were decreased, there was no change in the specific activity of the enzyme. These limited effects of a decrease in the dietary content of non-sulfur containing amino acids contrast with the more marked differences which we noted in studies which compared rats fed diets containing varying amounts of casein (2). One possible explanation was the constancy of the methionine content in the current experiments.

Effect of Dietary Methionine. We tested the effect of changes in the dietary content of methionine in a series of experiments in which we used basal diets which differed in their content of amino acids and/or choline. As shown in Table II, there was a consistent pattern of changes. In each of the three series the hepatic betaine decreased as the dietary content of methionine increased. The changes in the activity of betaine-homocysteine methyltransferase were biphasic. The addition of 0.3% methionine to a methionine-free diet resulted in a marked fall in the level of the enzyme in rat liver. The change was significant when we expressed enzyme activity as U/mg protein, U/g liver, or total hepatic units/g body weight. The magnitude of the decrease may be a function of the amino acid and choline contents of the basal diets and, for the specific activities, varied from -41% in animals fed the 5% amino acid -0.2% choline diets to -70% in rats fed the 22% amino acid -0.2% choline diets. In

TABLE II
EFFECT OF DIETARY METHIONINE ON BETAINE AND BETAINE-HOMOCYSTEINE METHYLASE IN RAT LIVER

Diet Components			Betaine	Betaine Methyltransferase		
AA	Choline %	Met	μmol/g	U/mg protein	U/g liver	U/liver/g BW
5	0.2	0	11.5±3.5	68±17	6589±1752	226±70
5	0.2	0.3	2.8±0.3[b]	40±3[b]	3372±268[b]	156±8[b]
5	0.2	1.0	0.9±0.1[a]	51±11	4156±815	193±24[c]
22	0	0	3.6±1.8	65±7	6339±1071	236±29
22	0	0.3	1.1±0.8[c]	28±6[a]	2799±979[b]	145±14[b]
22	0	1.0	0.5±0.5	48±9[b]	8498±1456[a]	288±71[b]
22	0.2	0	17.5±7.7	110±27	11537±2845	473±141
22	0.2	0.3	4.5±0.7[b]	33±7[a]	3208±690[a]	162±35[b]
22	0.2	1.0	0.5±0.8[c]	43±6[c]	4058±480[c]	209±14[b]

Each group consisted of at least five rats which were fed the designated diet for seven days.

P value for comparison with next lowest methionine intake:
[a] $P<0.001$; [b] $P<0.01$; [c] $P<0.05$

contrast, the supplementation of the 0.3% methionine diets with an additional 0.7% methionine resulted in an increase in hepatic betaine-homocysteine methyltransferase. The magnitude of the change in enzyme activity was affected by the means selected for the expression of the results as well as by the composition of the basal diet. The most marked changes were seen in the livers of rats fed the choline-free diet which contained 22% amino acids. The increases were +75%, +201% and +99% when calculted as U/mg protein, U/g liver, and total hepatic units/g body weight.

The data in Table II also dissociate the changes in enzyme activity from the changes in betaine content. In a recent, unpublished study we have found that the administration of betaine does increase hepatic betaine-homocysteine methyltransferase. Clearly the effect of dietary methionine is not mediated by a change in the level of this metabolite.

We have not defined the mechanism for the differences in the effect of methionine which are determined by the level of this amino acid in the antecedent diet. Betaine homocysteine methyltransferase is inhibited by both methionine and dimethylglycine (7). However studies with dialyzed

extracts failed to demonstrate the presence of soluble inhibitors. The existence of different isoenzymes might provide an explanation. One, present in the absence of adequate dietary methionine, could be repressed by the feeding of the amino acid. A second might be induced in the livers of rats fed the excessive (1.0%) methionine diets. To date we have found no evidence for different forms of betaine-homocysteine methyltransferase.

The results of the present studies warrant a revision of our previous conclusion concerning the metabolic role of the betaine-homocysteine methyltransferase reaction. During periods of excessive intake of methionine, this enzyme may serve both as a means for the catabolism of choline and for the removal of homocysteine. However, conservation of methionine may be an essential function of the betaine enzyme in animals fed diets inadequate in methionine. This role is consistent with the increased level of the enzyme in the livers of animals maintained on such diets. Furthermore, it is also consistent with the low K_m for homocysteine which is two orders of magnitude less than that with cystathionine synthase (1). Finally, impairment of this function by choline (and therefore betaine) deprivation, would explain both the observed fall in hepatic methionine (3) and the ability of folate + homocysteine to sustain only subnormal growth in rats fed a low methionine diet (8).

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