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Diet-induced changes in hepatic betaine-homocysteine methyltransferase activity are mediated by changes in the steady-state level of its mRNA

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Liver betaine-homocysteine methyltransferase (EC 2.1.1.5) activity fluctuates with changes in the dietary intake of sulfur amino acids, choline, and betaine. The purpose of this study was to determine whether dietary-induced changes in the activity of hepatic betaine-homocysteine methyltransferase are mediated by changes in the level of its mRNA. The hepatic activity and mRNA content of betaine-homocysteine methyltransferase were measured in rats fed one of five amino acid–defined diets: basal (1 g/kg methionine, 3 g/kg cystine, 1.25 g/kg choline bitartrate), control (basal plus 2 g/kg methionine), betaine supplemented (basal plus 3 g/kg betaine), cystine supplemented (basal plus 3 g/kg cystine), or betaine and cystine supplemented (basal plus 3 g/kg betaine plus 3 g/kg cystine). The basal diet was deficient solely in methionine, and the control diet was adequate in all nutrients. When compared with rats consuming the control diet, rats fed the methionine-deficient diet exhibited a 4-fold increase in the steady-state level of betaine-homocysteine methyltransferase mRNA ($p < 0.05$). Betaine addition to the methionine-deficient diet elevated mRNA level even further, resulting in a nearly 10-fold higher mRNA levels compared with the methionine-adequate control diet ($p < 0.05$). Dietary cystine had no effect on betaine-homocysteine methyltransferase mRNA levels. Liver betaine-homocysteine methyltransferase activity mirrored its mRNA levels. We conclude that dietary-induced changes of liver betaine-homocysteine methyltransferase activity are mediated by changes in the steady-state levels of its mRNA. (J. Nutr. Biochem. 8:541–545, 1997) © *Elsevier Science Inc. 1997*

Keywords: betaine-homocysteine methyltransferase; betaine; methionine; homocysteine; rats

Introduction

Betaine-homocysteine methyltransferase (BHMT) (EC 2.1.1.5) catalyzes a methyl transfer from betaine to homocysteine (Hcy), forming dimethylglycine and methionine (Met), respectively. The enzyme is abundant in mammalian liver, as well as in the kidney of some species. BHMT is required for the catabolism of betaine, and thus the complete oxidation of choline is linked to the availability of Hcy. There is significant interest in the regulation of Hcy and Met metabolism because elevated levels of plasma total homocyst(e)ine have been reported to be an independent risk factor for the development of arteriosclerotic vascular disease.[1] Plasma total homocyst(e)ine refers to the sum of all oxidized and reduced forms of Hcy found in blood plasma. The significance of choline oxidation and the BHMT catalyzed reaction to the overall regeneration of Met from Hcy remains speculative.

Liver BHMT activity has been shown to fluctuate with changes in the dietary intake of sulfur amino acids, choline, and betaine in rats[2–4] and chickens,[5] but the molecular

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Table 1 Growth performance and hepatic BHMT activity and mRNA content in rats fed Met-deficient amino acid–defined diets containing added Met, cystine, or betaine

Dietary treatment	Gain (g)	Gain: food intake (g/kg)	BHMT activity (U/mg)[2]	BHMT mRNA (rel)[3]
1. Basal (Met deficient)[1]	24[b]	240[a]	102[a]	4.8[a]
2. As 1 + 2 g/kg L-Met (control)	85[a]	467[b]	27[b]	1.0[b]
3. As 1 + 3 g/kg betaine	25[b]	225[a]	226[a]	8.7[a]
4. As 1 + 3 g/kg L-cystine	22[b]	240[a]	96[a]	4.1[a]
5. As 3 + 4	23[b]	230[a]	239[a]	10.4[a]
Pooled SEM	2	10	7	0.8

Data are means of seven rats fed the experimental diets for 14 days; average initial weight was 45 g. Means with unlike superscript are significantly ($p < 0.05$) different. [1]Diets were formulated based on AIN-93G recommendations for an L-amino acid–defined diet except for the content of choline, Met, and cystine. The basal diet contained the following levels of these nutrients: L-Met (1 g/kg), L-cystine (3 g/kg), and choline bitartrate (1.25 g/kg); it was devoid of betaine. [2]Units represent the nanomole product formed per hour per milligram protein. [3]Relative to GAPDH gene expression. The BHMT:GAPDH ratio of the control group (treatment 2) was assigned a relative value of 1.

events that mediate these responses have not been elucidated. We recently isolated cDNAs encoding porcine and human BHMT.[6] The data reported herein show that dietary-induced changes of liver BHMT activity are mediated by changes in the steady-state levels of its mRNA.

Methods and materials

Materials

[^{14}C-methyl]-betaine chloride (55 mCi/mmol) was a gift from Dr. James Finkelstein (VA Medical Center, Washington DC). [α-^{32}P]dCTP (3000 Ci/mmol) and a random prime DNA labeling kit were obtained from Amersham Life Sciences Incorporated (Arlington Heights, IL). Betaine hydrochloride was purchased from Sigma Chemical Company (St. Louis, MO). L-Met, L-cystine, choline bitartrate, and a pre-mixed L-amino acid–defined diet devoid of these nutrients were purchased from Dyets (Bethlehem, PA). All other reagents were of the highest purity commercially available.

Diets and animal protocol

The level of nutrients in the amino acid–defined diets used in this study, except for Met, cystine, and choline bitartrate, were at the levels recommended for the growing rat as defined by the American Institute of Nutrition (AIN-93G).[7] The levels of Met, cystine, and choline bitartrate used in this study were 1 or 3 g/kg Met, 3 or 6 g/kg cystine, and 1.25 g/kg choline bitartrate. The level of these nutrients in the AIN-93G amino acid–defined diet are 4.5 g/kg Met, 3.7 g/kg cystine, and 2.5 g/kg choline bitartrate. Where indicated, betaine was added as the chloride salt to provide a level of the free base at 3 g/kg diet. The small variations in the total sum of dietary amino acids and betaine among diets were compensated for by displacing an equivalent amount of microcrystalline cellulose. The diets used in this study are summarized in *Table 1*.

The level of choline used in this study, although lower than current AIN-93G recommendations, has previously been shown to prevent the development of fatty liver when rats are fed a Met-deficient diet.[8,9] The control level of sulfur amino acids used were those that have been shown to be the minimum levels required to support maximum growth of weanling rats fed a chemically defined amino acid diet,[10] i.e., 3 g/kg Met and 3 g/kg cystine. Therefore, the basal diet (treatment 1) was solely deficient in Met (33% of requirement), whereas the control diet (treatment 2) contained adequate levels of all nutrients. Dietary treatments 3, 4, and 5 were deficient in Met and had either adequate (3 g/kg) or surfeit levels (6 g/kg) of cystine and were either devoid or contained 3 g/kg of betaine.

Three-week-old Sprague Dawley rats (Harlan, Indianapolis, IN) were housed individually in hanging stainless-steel cages in a light (12 h light/12 h dark)- and temperature (23°C)-controlled room. All rats were fed the Met-adequate control diet for 3 days to allow adaptation to the purified diet. Following the adaptation period, rats were divided into five groups of seven rats such that mean body weights among groups ($n = 7$) were not significantly different.[5] Each group was given free access to water and one of the experimental diets throughout a 14-day feeding period.

On the last morning of the feeding trial, food cups were removed for 3 h, after which the rats were killed by asphyxiation with carbon dioxide. The livers were rapidly removed and frozen in liquid nitrogen; they were then stored at −80°C until subsequent isolation of total RNA and measurement of BHMT activity was performed.

This study was approved by the University of Illinois' Laboratory Animal Care Advisory Committee.

Assay procedures

Northern blot analysis was performed using standard procedures. Total RNA was isolated using the Ultra-SpecII RNA isolation system (Biotecx, Houston, TX). Twenty micrograms of each RNA sample was size-separated using morpholinopropanesulfonic acid (MOPS)–formaldehyde–agarose electrophoresis. The RNA was then transferred onto nylon membranes (U.S. Biochemicals, Cleveland, OH) using the capillary transfer procedure described by Sambrook et al.[11] The RNA was UV cross-linked to the membrane and probed using oligonucleotides generated by a random-priming procedure using the porcine BHMT cDNA[6] or rat liver glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (EC 1.2.1.12) cDNA as template. Prehybridization, hybridization, and washing buffers were prepared as previously described by Church and Gilbert.[12] The hybridization temperature used for the detection of both mRNAs was 65°C. Band intensity was quantified using a Molecular Dynamics 425S PhosphorImager (Sunnyvale, CA), and BHMT mRNA levels were normalized relative to GAPDH mRNA abundance so that the BHMT/GAPDH mRNA ratio of the Met-adequate control group was equal to 1. RNA sizes were estimated by comparison to a 0.24–9.5 kb ladder of synthetic RNA (Life Technologies, Inc., Grand Island, NY).

BHMT activity was measured in crude liver extracts as previously described,[6] with the exception that livers were homogenized in 5 volumes of buffer. All assays were initial-rate measurements using saturating levels of substrate concentrations: 2 mM betaine (0.1 μCi) and 5 mM DL-Hcy. Each sample was assayed in duplicate, and duplicate measurements varied less than 3%. No more than 8% of the limiting substrate was consumed in any assay. Total protein in crude liver extracts were measured by the method of Bradford (Bio-Rad Laboratories, Hercules, CA). A unit of BHMT activity is defined as a nanomole of Met formed per hour.

Statistics

Data were analyzed by one-way analysis of variance. When analysis gave a significant F value ($p < 0.05$), treatment differences were evaluated using Fisher's least-significant difference procedure.

Results

Rats fed the Met-deficient diets (treatments 1, 3, 4, and 5) consumed 40% less food (not shown) and gained less weight (*Table 1*) than rats fed the control diet containing adequate Met (treatment 2). The gain-to-food intake ratios indicated that the basal level of Met was severely deficient, although all rats gained weight, indicating that the amount of Met consumed was above the maintenance requirement for the weanling rat. Dietary additions of betaine or cystine had no effect on growth performance.

Based on previous reports,[2–5] we expected the dietary treatments employed in this study to result in a wide range of hepatic BHMT activities, and as shown in *Table 1*, we did observe a large range of activity. Rats fed the Met-deficient basal diet had BHMT activities that were approximately 4-fold higher ($p < 0.05$) than the rats fed the Met-adequate control diet. Betaine supplementation of the Met-deficient diets (treatments 3 and 5) resulted in a further increase ($p < 0.05$) in BHMT activity relative to the rats fed diets solely deficient in Met (treatments 1 and 4). Rats fed the Met-deficient diets with supplemental betaine had greater than an 8-fold higher BHMT activity than rats fed the Met-adequate control diet. In contrast to previous reports,[4,5] dietary cystine had no effect on BHMT activity.

Liver BHMT and GAPDH gene expression were quantified by phosphorimaging Northern blots (*Table 1*). The data for BHMT gene expression are expressed relative to GAPDH mRNA levels. In brief, the relative level of BHMT gene expression mirrored BHMT activity. *Figure 1* shows an autoradiograph of a Northern blot of BHMT and GAPDH mRNA transcripts from one rat chosen at random from each dietary treatment. The autoradiograph shows that the relative levels of BHMT gene expression were markedly affected by the dietary treatments; they also were consistent with the data obtained by phosphorimaging. Only one band was detected when blots were probed for either BHMT or GAPDH mRNA. Rat liver BHMT mRNA was estimated to be 2.1 kb, similar in length to the 2.4 kb pair human cDNA.[6]

Discussion

BHMT is expressed primarily in the liver of rats,[13] and previous studies have shown that the specific activity of this enzyme varies with the dietary intake of sulfur amino acids, choline, and betaine.[2–4] The greatest changes have been observed with Met deficiency and with Met deficiency in combination with excess dietary choline. Up to 3-fold variations of BHMT activity were observed. Similar changes, albeit greater in magnitude, were observed for BHMT activity in chicken liver when chicks were fed dietary levels of sulfur amino acids and betaine similar to those used herein.[5] Our study here is the first to show the effects of these dietary patterns on BHMT gene expression.

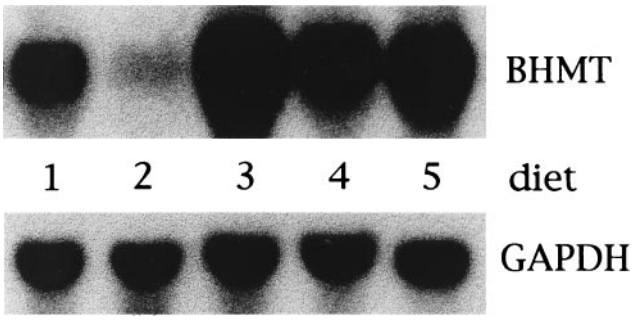

Figure 1 Diet-induced changes of hepatic BHMT gene expression. Rats were fed experimental diets for 14 days as follows: 1, Met-deficient basal diet; 2, Met-adequate control diet; 3, basal diet plus supplemental betaine; 4, basal diet plus supplemental cystine; and 5, basal diet plus supplemental betaine and cystine. Liver RNA was isolated and probed for BHMT and GAPDH mRNA levels by Northern blot analysis. The figure shows a representative sample from each dietary treatment as visualized by autoradiography. The autoradiographic image was captured using the Foto/Analyst II Visionary System and Collage software (Fotodyne, New Berlin, WI).

We hypothesized that the diet-induced changes in BHMT activity are probably not caused by changes in the concentration of some known or unknown effector(s) of the enzyme. For example, the deduced amino acid sequence of human BHMT includes a putative S-adenosylmethionine binding site,[6] and S-adenosylmethionine and S-adenosylhomocysteine have been reported to be inhibitors of BHMT activity.[14,15] However, our extract preparation and assay procedure resulted in a 150-fold dilution of metabolite concentrations, and it seems unlikely that a cell would normally accumulate an effector 150 times higher than that required for activation or inhibition of a target enzyme. A 150-fold dilution of the S-adenosylmethionine and S-adenosylhomocysteine concentrations normally present in rat liver would not approach the level that was reportedly required to inhibit the rat enzyme in vitro. Furthermore, mixing individual liver extracts prepared from rats consuming the control (treatment 2) or betaine-supplemented diets (treatments 3 and 5) resulted in BHMT-specific activities that equaled the mean of the specific activities obtained when the individual extracts were assayed alone (not shown). This observation indicates that the differences observed in BHMT activity among the experimental groups were not due to any soluble factor present in one extract and not the other. Although it is possible that changes in BHMT activity could result from an uncharacterized post-translational covalent modification of the enzyme or increased translation of its mRNA, we decided to investigate whether changes in liver BHMT activity were caused by changes in steady-state mRNA levels.

To investigate nutrient effects on BHMT gene expression, we prepared amino acid–defined diets that varied in sulfur amino acid and betaine content. Based on the previous studies of Finkelstein et al. with rats[2–4] and our own recent study with chickens,[5] we expected the diets prepared for this study to cause a significant variation in liver BHMT activity. As can be seen in *Table 3*, nearly 9-fold differences in BHMT activity were observed. We did not include a choline-supplemented group because choline and betaine supplementation have been shown to have similar effects on BHMT activity in chickens.[5] Both chickens and rats express very high levels of choline oxidase in liver.[16] Northern blot analysis of rat liver total RNA was used to monitor BHMT gene expression. The activity and Northern blot profiles indicated that the dietary-induced changes in BHMT activity were mediated by changes in steady-state levels of its mRNA (*Table 1* and *Figure 1*).

It has been shown that rats reduce their food intake when offered diets restricted in one of the essential amino acids.[17] Consistent with previous observations, we observed a 40% reduction of food intake when rats consumed the Met-deficient diets. The data reported here do not eliminate the possibility that some of the changes in mRNA and activity levels of BHMT were due to a physiological response related to reduced food intake rather that Met deficiency per se. Rats consuming the Met-deficient basal diet (treatment 1) had higher hepatic BHMT activity and mRNA levels than those consuming the Met-adequate control diet (treatment 2). However, the further elevation of BHMT mRNA and activity in rats consuming the basal level of Met with supplemental betaine (treatments 3 and 5), compared with rats consuming the basal level of Met with or without supplemental cystine (treatments 1 and 4), were independent of food intake, because all of these Met-deficient diets caused similar decreases in food intake.

We recently showed that chicken liver BHMT activity was markedly increased ($p < 0.05$) when a Met-deficient diet containing excess betaine was supplemented with excess cystine. The rats used herein, however, did not respond in this manner to cystine. Whether such an effect would be observed in rats consuming greater than two times their requirement for cystine is not known.

Whether the elevated levels of BHMT activity observed in rats consuming Met-deficient diets with added betaine (treatments 3 and 5) would result in greater flux of Hcy to Met is problematic. The fact that the betaine-supplemented rats did not grow faster than those fed the basal diet indicates that if net Hcy remethylation was indeed increased by betaine, the additional Met formed must be preferentially channeled into S-adenosylmethionine-dependent pathways before it was used for net protein accretion. It has been shown that independent of dietary choline, Met deficiency increases liver betaine concentrations.[18] This rise in liver betaine could be caused by enhanced oxidation of choline to betaine, by reduced demethylation of betaine via the BHMT reaction, or by a combination of both. Although we did not measure hepatic betaine and Hcy levels, previous studies[2,18–20] indicate that rats fed diets devoid of choline and betaine have liver betaine levels in excess of 500 μM, a level that would saturate BHMT since the K_m of betaine for the rat enzyme is 48 μM.[21] Even though the level of BHMT increases so dramatically under conditions of Met deficiency, the low level of Hcy normally found in rodent liver, reportedly 4 μM,[22–24] is below the K_m for rat BHMT.[21] The level of Hcy may be even lower under conditions of Met deficiency, thus limiting betaine metabolism. Intraperitoneal injection of Hcy in rats consuming low protein diets have been shown to lower liver betaine concentrations.[18] Taken together, these data indicate that the concentration of hepatic Hcy in the rat is rate limiting for the BHMT reaction under all physiologically relevant dietary conditions.

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