Isolated Hypermethioninemia: Measurements of *S*-Adenosylmethionine and Choline

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The concentrations of methionine and *S*-adenosylmethionine (AdoMet) in plasma and free choline and phospholipid-bound choline in both plasma and red blood cells from individuals with isolated hypermethioninemia have been measured. The only genetic abnormalities identified in these individuals have been inactivating mutations in *MAT1A,* the gene that encodes the subunit of the isozymes of methionine adenosyltransferase (MAT), MAT I, and MAT III, expressed only in adult liver. These measurements were performed to learn more about AdoMet metabolism and to test the working hypotheses that inadequate delivery of AdoMet, or of choline or a choline derivative, from liver to brain might be a cause of the neurologic disease often found in humans with the most severe losses of MAT I/III activity. In striking contrast to the elevations of plasma AdoMet reported in control humans with hypermethioninemia resulting from methionine loading, plasma AdoMet levels were generally below the mean reference value in the MAT I/III-deficient hypermethioninemic patients. This is interpreted as a result of subnormal formation of AdoMet in liver due to the deficient activity of MAT I/III and resultant lower-than-normal delivery of AdoMet from liver to plasma. A low plasma AdoMet concentration in the presence of an elevated methionine provides a useful diagnostic tool that pinpoints the cause of a case of hypermethioninemia as defective MAT I/III activity. Plasma-free choline concentrations were also generally somewhat below normal in the hypermethioninemic patients. However, neither plasma AdoMet nor plasma choline concentrations were strikingly lower in MAT I/III-deficient individuals with neurologic abnormalities than in those without. These results thus fail to provide support for the working hypotheses in question.

THE TERM ISOLATED persistent hypermethioninemia designates abnormally elevated plasma methionine that continues beyond early childhood and is not associated with homocystinuria due to cystathionine β-synthase deficiency, tyrosinemia type I, or severe liver disease. Since 1974, more than 60 individuals with this abnormality have been described.[1-18] Early assays of crude liver extracts often showed that hypermethioninemic individuals had deficient activity of methionine adenosyltransferase (MAT) in this organ.[1,4,8,10,19] MAT activities were normal in cultured skin fibroblasts, red blood cells, or lymphoid cells.[4,20,21] Subsequent molecular genetic studies confirmed and extended these results: in all subjects in whom the underlying mutations have been identified, the hypermethioninemia is due to inactivating mutations in *MAT1A,*[15,17,22-25] the gene that encodes the subunit, that, as tetramers and dimers, form the 2 MAT isozymes, MAT I and MAT III, expressed solely in adult mammalian liver.[26] The existence of a separate gene, *MAT2A,* encoding the catalytically active subunit of another MAT isozyme, MAT II, expressed as far as is known in all nonhepatic cells, fetal liver, and to a slight extent, even in adult liver,[26] explains the normal MAT activities found in tissues other than liver of individuals now known to be MAT I/III-deficient. That defects in the MAT-dependent conversion of methionine to *S*-adenosylmethionine (AdoMet) lead to hypermethioninemia is in accord with other evidence that this step is the first in the major pathway by which mammals catabolize methionine.[18] Flux through the most important known alternative pathway for methionine degradation, initiated by transamination to 4-methylthio-2-oxo-butyrate, increases as plasma methionine increases, but only after plasma methionine reaches concentrations of 300 to 350 μmol/L, almost 10 times the normal upper limit of this concentration.[14]

Clinically, the majority of MAT I/III-deficient individuals have apparently been unaffected, but among those with the more severe losses of activity, as judged by the type of mutation, there has been a clustering of neurologic or cognitive problems.[17] For example, of 4 patients with proven homozygous truncating mutations in *MAT1A* that, when tested, are devoid of MAT I/III activity in expression systems, 2 have cerebral demyelination,[12,14,23] but 2 have normal myelination.[17,25] In the only such patient to be treated with AdoMet, magnetic resonance imaging (MRI) evidence of demyelination was reversed during therapy.[12] Brain demyelination was also found in a patient homozygous for a splicing defect in *MAT1A.* Because this individual has extreme elevations of plasma methionine comparable to those found in patients homozygous for null mutations, he, too, may well have little or no MAT I/III activity.[14,17] Further indication that inadequate MAT activity may lead to abnormalities of myelin is provided by the observations that young rats treated with cycloleucine, a compound known to inhibit all 3 mammalian MAT isozymes,[27] develop such abnormalities, at least partially counteracted by treatment with AdoMet.[28]

The findings with the human subjects raise the questions of whether there might be a causal relationship specifically between especially severe MAT I/III deficiency and neurological problems and, if so, the mechanism of this relationship. These questions are especially interesting because in both

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bovine and rat brain only *MAT2A* is expressed,[29,30] and the kinetic properties of MAT activity in human brain support the likelihood that this is true also for the human brain.[18,31,32] The possibility arises of an unrecognized link between defective synthesis of AdoMet in liver and brain disease, despite normal activity of MAT in the latter organ. Among the potential explanations of such a link are the existence of export-import systems that normally deliver from the liver to the brain either AdoMet itself or a methylated compound synthesized chiefly in liver in an AdoMet-dependent reaction.[23] Among the many products formed by the action of AdoMet-dependent methyltransferases,[33] at least 39 of which are known in mammals,[34] the choline moiety is a prime candidate to play a role in brain myelination. This moiety is formed chiefly in liver by successive AdoMet-dependent methylations of phosphatidylethanolamine,[35-38] and choline derivatives in the form of phosphatidylcholine and sphingomyelin are major constituents of myelin. As a first step to test these possibilities and to learn more about AdoMet metabolism in isolated hypermethioninemia, we assayed concurrently methionine and AdoMet in plasma and free choline and phospholipid-bound choline in both plasma and red blood cells from individuals with isolated hypermethioninemia, most of whom have defined mutations in *MAT1A.* The results are reported here.

PATIENTS AND METHODS

Patients

The patients each have persistent isolated hypermethioninemia, as documented previously. Patient identifiers that permit comparison with previously published information about them are included in Table 1.

Methods

Plasma methionine was measured by column chromatography using a Beckman 6300 Amino Acid Analyzer (Beckman Instruments, Palo Alto, CA). Plasma AdoMet was determined as its fluorescent isoindole derivative.[39] Plasma- and red blood cell-free and phospholipid-bound choline were assayed in duplicate as described.[40,41] Plasma samples were stored at −20°C until assayed. AdoMet has been found to be stable in plasma stored in this manner for as long as 2 years. For the subjects reported here, samples were stored for only a few days or for as long as 13 months before analysis. Occasionally, for unknown reasons, AdoMet was lost from samples stored for less than 2 years, but such artefactual decreases were apparent because they were much greater than 50%, and the AdoMet values from these samples could be disregarded. Red blood cell samples were stored at −70°C without prior deproteinization.

RESULTS AND DISCUSSION

Table 1 lists the hypermethioninemic patients studied (in descending order according to their plasma methionine concentrations) and provides citations to the articles reporting the most recent clinical information about them. Each of the 4 patients with the highest plasma methionine has neurologic abnormalities: patient 7 and patient C have brain demyelination[12,17,23]; patient 9 has an IQ in the lowest 1 percentile[17]; and patient 5 is mentally slow compared with other members of her family.[17] The remainder of the patients listed are free of clinical abnormalities. Table 1 also specifies the inactivating *MAT1A* mutations present in each subject (where known), the relative enzyme activity of the expressed mutations, and the publications in which these mutations are documented. Also listed are the measured concurrent concentrations of methionine and AdoMet in plasma and free choline and phospholipid-bound choline in both plasma and red blood cells. A wide range of elevations of plasma methionine was observed among these patients. Although it was not possible to be certain that each blood sample was collected under fasting conditions, a comparison of the 2 samples collected on successive days from Mr. C indicates that ingestion of a normal meal 3 hours before the sample was drawn made a negligible difference in the plasma methionine. This may be due to the fact that these hypermethioninemic patients carry such large body loads of (nonprotein) methionine (estimated, for example, in Mr C to be 154 mmol[21]) that ingestion of the methionine from a normal meal (perhaps 3 to 5 mmol) would cause only a minor percentage change. Similarly, although longer term, drastic limitation or increase in dietary methionine may lower or raise plasma methionine concentrations in hypermethioninemic patients,[14,21] the present subjects were on normal diets, so the plasma methionine concentrations observed should have been little affected by such dietary extremes.

It is not possible from the expression system measurements reported in Table 1 to specify with certainty the MAT activity contributed by a particular mutant allele in liver cells. Confounding effects, such as mRNA and/or enzyme stability, or alterations in subunit interactions cannot definitively be taken into account. Nevertheless, it appears that the extent of the abnormal elevations in plasma methionine in these patients most likely reflects to a greater or lesser degree the severity of the losses of MAT I/III activity in the patients. High elevations of plasma methionine were present in patients C, Mr C, and patient 3, each of whom were homozygous for either truncating *MAT1A* 351X or 185X, as well as in another girl (patient 8) homozygous for 350X.[23] Although a sample from this patient was not available for the present study, her previously reported plasma methionine concentrations ranged from 1,114 to 1,629 μmol/L.[14] 351X and 350X are devoid of activity when expressed, and although this has not been confirmed directly, it is assumed that even more severely truncated 185X is also devoid of activity. Generally milder elevations of plasma methionine occurred in patients with 2 proven point mutations (patients 13, 10, 11, 2, and 14). Although each of these point mutations is inactivating, each contributed some activity when expressed. Very mild elevation of plasma methionine was noted in patient CII-5 (and in all other subjects heterozygous for the R264H mutation[24]) in whom as much as 20% to 25% of normal MAT I/III activity would be expected due to formation of dimers and tetramers composed solely of the wild-type enzyme subunit encoded by the wild-type *MAT1A* allele present in these individuals. In view of this rough correlation of the extent of elevation of plasma methionine with the expected residual MAT I/III activity, it seems reasonable to postulate that patient 7 (homozygous for a splicing mutation, the effect of which on activity could not be evaluated by expression studies), patient 9 (a compound heterozygote for truncation 185X and point mutation R199C), and even patient 5 (a compound heterozygote for 2 point mutations) each has very severe loss of MAT I/III activity.

The mean plasma AdoMet concentration for control children (age 2 to 8 years) was 89.2 nmol/L with a standard deviation of ±17.9 nmol/L (range, 71.3 to 126 nmol/L; n = 9); for adults,

Table 1. Inactivating Mutations in *MAT1A* and Concurrent Measurements of the Concentrations of Plasma Methionine, AdoMet, Free Choline, and Phospholipid-Bound Choline, and Red Blood Cell–Free Choline and Phospholipid-Bound Choline in Patients With Isolated Hypermethioninemia

Patient No.[a] (reference)[b]	Mutation(s) Allele 1 (activity); Allele 2 (Activity)[c]	Age (yr)	Plasma Methionine (μmol/L)[d]	Plasma AdoMet (nmol/L)[e]	Plasma-Free Choline (μmol/L)[f]	Plasma- Bound Choline (μmol/L)[g]	Red Blood Cell-Free Choline (μmol/L)[h]	Red Blood Cell- Bound Choline (μmol/L)[i]
7[17]	292G ↓ gt → A ↓ gt (unk);	5.3	1,505	51.5 (2)[k]	10.59	2,107	53.5	1,656
7	292G ↓ gt → A ↓ gt (unk)[j]	6.3	1,394	93.7	6.85	NA[l]	20.5	NA
9[17,23]	185X; R199C[m] (11%)	6.0	1,265	87.4	7.29	NA	16.0	NA
5[17,23]	R264C (0.3%); G336R (23%)	12	1,199	64.5	5.25	1,800	14.7	1,290
Patient C[12,23]	351X (0%); 351X (0%)	19	795	70.9 (2)	6.84	3,220	41.7	1,480
Mr C[25]	185X; 185X	43[n]	793	60.9	9.74	2,104	100.4	1,901
Mr C		43[o]	792	63.5 (2)	7.89	2,167	102.4	1,536
3[14,23]	185X; 185X	6.5	618	48.2 (2)	7.67	1,662	23.5	1,562
13[14,23]	R199C (11%); R199C (11%)	6.2	520	88.5 (2)	10.18	3,206	40.8	1,657
10[14,23]	R199C (11%); R199C (11%)	8.4	488	77.5	9.39	2,320	23.0	1,110
11[14,23]	R199C (11%); R199C (11%)	12	479	75.8 (2)	9.83	2,030	17.8	1,280
2[p 14,22]	I322M (11%); I322M (11%)	24	346	76.0 (2)	5.46	2,844	117.1	1,511
18[14]	Unknown; unknown	2.8	258	89.2	12.21	1,988	48.6	1,469
14[14,17]	I322M (11%); E344A (12%)	6.4	206	88.7	9.80	2,134	88.8	1,390
CII-5[24]	Wild-type; R264H[q]	38	64	112	NA	NA	NA	NA
24[14]	Unknown; unknown	4.4	52	63.3	8.69	1,594	34.9	1,243

[a]Except where noted the patient had not necessarily been fasting overnight at the time the blood sample was drawn. Samples are listed in descending order of plasma methionine concentrations.

[b]The citations are: first, those in which the most recent available clinical information about the patient in question is reported; and, second, those in which the *MAT1A* mutation(s) in that patient and their activities in expression systems are detailed.

[c]The activities are listed as percent of wild-type activity when the mutation was expressed in the systems specified in the original report cited.

[d]Reference range, 23 to 45 μmol/L.

[e]Reference range, 92.8 ± 16.2 nmol/L (mean ± SD; n = 25) (see text).

[f]Reference range, 11.4 ± 3.7 μmol/L (mean ± SD).[42] Note that in the report cited,[42] this range, and those for the other choline-containing compounds, were erroneously described as ''mean ± standard error,'' rather than the correct ''mean ± standard deviation.''

[g]Phospholipid-bound choline: reference range, 2,364 ± 774 μmol/L (mean ± SD).[42]

[h]Reference range, 18.1 ± 10.4 μmol/L (mean ± SD).[42] The values reported by Buchman et al[42] were obtained with samples of red blood cells frozen without prior deproteinization, as were the samples used in the present study. Free choline in such red blood cell samples was found by Miller et al[43] to increase by a mean of 1.42 times above the values measured in unfrozen cells.

[i]Phospholipid-bound choline: reference range, 1,667 ± 331 μmol/L (mean ± SD).[42]

[j]Splicing mutation: resultant effect on enzymic activity unknown.

[k]Value listed is the mean of 2 duplicate determinations.

[l]NA, not analyzed.

[m]Mutations are specified as illustrated by the following examples: 185X = premature truncation at amino acid residue 185; R199C: a nucleotide change that causes replacement of arginine 199 by cysteine.

[n]Blood sample drawn 3 hours after the patient had eaten breakfast.

[o]Fasting sample.

[p]Patient 2[14] is patient 1 of Gaull et al[1] (referred to as G1 by Ubagai et al[22]).

[q]MAT1A*R264H is a point mutation that causes mild hypermethioninemia inherited in a Mendelian dominant mode.

95 ± 15.5 nmol/L (range, 71 to 125 nmol/L; n = 16). These means did not differ significantly. The overall group mean was 92.8 ± 16.2 nmol/L (n = 25). The concentrations of plasma AdoMet in the hypermethioninemic subjects were usually below this group reference mean value, with a few falling more than 2 standard deviations below, and the mean for the patients, 75.7 ± 16.9 nmol/L (n = 16) was statistically significantly below the group mean (2-tailed *P* value = .0022). These results are in striking contrast to those reported for normal human subjects with elevated plasma methionine concentrations after oral loads of methionine: Loehrer et al[44] reported that after an oral dose of methionine of 0.1 g (0.67 mmol)/kg body weight, the mean AdoMet plasma concentration of control human subjects increased to a peak some 8 hours later equal to 6.3 times the preload value. Although concentrations of methionine in plasma were not reported by these investigators, Boers et al[45] found that after similar loads to control subjects, plasma methionine peaked after 1 hour in 1 study to a mean of 942 μmol/L and in a second study peaked at 1,033 μmol/L in premenopausal women and 1,063 μmol/L in men.[46] Capdevila and Wagner[39] observed that after a similar load plasma methionine in a control subject increased from 34 μM to 816 μM at 3 hours, and plasma AdoMet increased from 99 nmol/L to peak at 381 nmol/L at 9 hours. The kinetic properties of MAT I/III and MAT II are such that liver is the only organ in which a marked increase in the concentration of AdoMet occurs after a methionine load.[18,47,48] Loehrer et al[44] suggested, therefore, that the increase in plasma AdoMet after the methionine load ''probably reflects liver metabolism.'' The present results strongly support this conclusion because most of the hypermethioninemic pa-

tients studied are known to have deficient activity of MAT I/III in liver, but are proven[12,21] or may be presumed to have normal MAT II activity in other tissues.

To gain further assurance that the AdoMet-like material observed in the MAT I/III-deficient patients was indeed AdoMet and not some contaminating material, advantage was taken of the known heat-lability of AdoMet. Aliquots of the plasma samples from patients 2, 3, 5, 7, 10, 11, 13, C, and Mr C were heated for 6 minutes in a boiling water bath. In each instance, as had occurred also with samples from normal control subjects,[39] the AdoMet peak was completely removed with no fluorescent material eluting in the same position.

The failure of the patients studied to have elevated plasma AdoMet in the face of their elevations of plasma methionine can be attributed to a deficit in these patients in the capacity of increased methionine to promote increased hepatic synthesis of AdoMet. Lack of elevation of plasma AdoMet in the presence of elevated plasma methionine then becomes a useful diagnostic tool that indicates the cause of a particular case of isolated hypermethioninemia is deficient MAT I/III activity. Cases of isolated hypermethioninemia have been reported in which the available evidence suggests liver MAT activity is normal[5,18] or associated with abnormalities of fatty acid metabolism.[6,16] It would be of interest to assay plasma AdoMet in such patients.

The AdoMet present even in the plasma of patients with homozygous truncating mutations in *MAT1A* (patients C, Mr C, and 3) must be formed by the action of MAT II. Both patient C and Mr C have normal activity of *MAT2A*-encoded MAT II.[12,21] Balance studies showed that Mr C does form an amount of AdoMet just sufficient to meet his needs.[21] The distribution of such MAT II-dependent synthesis between tissues other than liver, and liver itself, is uncertain. MAT II is expressed in hepatocytes to a small extent and more plentifully in endothelial and Kupffer cells of the liver.[49] Because the MAT I/III-deficient patients generally had somewhat low plasma concentrations of AdoMet, it appears probable that the action of MAT II alone is usually not sufficient to maintain the normal plasma concentration of AdoMet, and that AdoMet formed by MAT I/III in the liver must also contribute to plasma AdoMet, even when the plasma concentration of methionine is normal.

Thus, with respect to the possibility that defective export of AdoMet from the liver may be causative of brain demyelination, the present evidence strengthens the likelihood that such export does occur. A carrier-mediated nucleoside transport system has recently been described in rat brain endothelial cells that has an affinity for AdoMet in the same range as the K_m for thymidine and other substrates.[50] In addition, hypermethioninemic patient C, who developed brain demyelination, had an abnormally low concentration of AdoMet in cerebrospinal fluid despite abnormally elevated methionine in both plasma and cerebrospinal fluid. As mentioned above, she had a normal activity of MAT II in red blood cells[12] (and therefore, presumably in brain) and is homozygous for a truncating mutation in *MAT1A*. Her low cerebrospinal fluid AdoMet would be explained parsimoniously by deficient transport of AdoMet from liver to brain. Despite these lines of evidence compatible with the existence of a normally occurring, physiologically important, transport of AdoMet from liver to brain, the present measurements of plasma AdoMet fall short of providing compelling indication that a deficit in such transport causes the neurologic abnormalities noted among MAT I/III-deficient patients with the most severe lack of this activity. Thus, the plasma concentrations of AdoMet among the 4 individuals in Table 1 who had central nervous system (CNS) disease (patients 7, 9, 5, and C) were not strikingly lower than the concentrations of this compound in plasma of the other hypermethioninemic subjects free of such abnormalities.

An alternative hypothetical cause of brain demyelination in MAT I/III-deficient patients postulates defective transport from liver to brain of choline or some choline derivative. A major route for de novo synthesis of the choline moiety in mammals involves the AdoMet-dependent methylation of phosphatidylethanolamine, catalyzed by a methyltransferase found in abundance in hepatocytes, but expressed only minimally in other cells and tissues.[35-38] Recent experiments with mice, in which the gene encoding this enzyme has been disrupted, convincingly establish that under conditions of inadequate dietary intake of choline, the action of this enzyme is essential.[51] Phosphatidylcholine synthesized in the liver is exported to the serum[52,53] where it may be degraded to choline.[52] Phosphatidylcholine is also a precursor in the biosynthesis of sphingomyelin.[54] Although there has been some controversy as to whether significant amounts of choline are taken up into the brain in lipid-bound forms,[55-57] it has now been clearly established that brain uptake of free choline does occur via a specific, energy-dependent transport system.[58,59] The K_m of this system both in experimental animals and in man is thought to be poised close to the normal concentration of free choline in the plasma, so that small increases in the free choline concentration in plasma may change the balance of brain uptake from negative to positive.[60] Thus, in normal humans the arteriovenous difference in choline concentrations across the brain shows that choline is lost from the brain after 18 hours of fasting,[61] whereas after choline ingestion, choline is taken up by the brain.[62] Given this delicate balance, it might be that inadequate AdoMet-dependent hepatic synthesis of phosphatidylcholine might chronically reduce the plasma concentration of free choline enough so that brain uptake would not be sufficient to produce and maintain normal myelination.

The data for free choline and phospholipid-bound choline reported in Table 1 indicate that free choline was generally somewhat below the reference mean in the hypermethioninemic patients. However, the patients who developed demyelination did not have markedly lower plasma concentrations of these compounds than did the patients who did not develop demyelination. Perhaps dietary intake of choline and choline derivatives is sufficient to maintain the plasma concentrations of these compounds at not too abnormally low levels, a possibility compatible with the observation that mice completely lacking phosphatidylethanolamine methyltransferase activity were clinically unaffected when fed a standard laboratory diet.[51]

The values for erythrocyte-free choline in some subjects appear to fall above the reference range. However, the distribution of this variable in the normal population is markedly skewed toward higher concentrations (Jenden, unpublished data). Several investigators have pointed out that high values

may be seen with unusual frequency in various illnesses (see references cited by Jope and Jenden[63]), and extremely high values may persist for several weeks after the administration of lithium[63] (although to the best of our knowledge, none of the patients reported on in this article had been treated with lithium). These observations suggest that high levels of free choline in erythrocytes may occur with significant frequency in subjects who may not be recognizably abnormal in other ways. Nor do the erythrocyte free choline concentrations among the listed patients with neurologic abnormalities differ strikingly from those in the patients free of such difficulties.

In summary, the data reported fail to provide compelling support for the possibilities that a complete lack of MAT I/III activity decreases delivery of either AdoMet or choline (or a choline derivative) from liver to brain sufficiently to provide an explanation for the neurologic abnormalities that have been observed in several patients with such genetic defects. However, because it has been possible to study only a few such patients and because the studies were necessarily performed after the neurologic problems were already present, in our opinion, these working hypotheses have not been completely ruled out. Further studies, perhaps focusing also on other possible candidate compounds formed in AdoMet-dependent reactions in the liver and delivered to the brain, are needed.

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