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S-ADENOSYL-L-HOMOCYSTEINE IN BRAIN
Regional Concentrations, Catabolism, and the Effects of Methionine Sulfoximine[1]

ROBERT A. SCHATZ, CHOUDA RANI VUNNAM, AND OTTO Z. SELLINGER
Laboratory of Neurochemistry
Mental Health Research Institute
University of Michigan Medical Center
Ann Arbor, Michigan 48109

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Administration of methionine sulfoximine (MSO) to rats and mice significantly decreased cerebral levels of *S*-adenosyl-L-homocysteine (AdoHcy). Concurrent administration of methionine prevented this decrease and, when methionine was given alone, significantly elevated AdoHcy levels resulted in both species. Regionally, AdoHcy levels varied from 20 nmol/g in rat cerebellum and spinal cord to about 60 nmol/g in hypothalamus and midbrain. MSO decreased AdoHcy in all regions tested except striatum, midbrain, and spinal cord. AdoMet/AdoHcy ratios (methylation index) varied from 0.48 in hypothalamus to 2.4 in cerebellum, and MSO administration decreased these ratios in all regions except hypothalamus. AdoHcy hydrolase activity was lowest in hypothalamus, highest in brainstem and, generally, varied inversely with regional AdoHcy levels. MSO decreased AdoHcy hydrolase activity in all regions except hypothalamus and spinal cord. Cycloleucine administration resulted in significantly decreased levels of mouse brain AdoHcy, whereas the administration of dihydroxyphenylalanine (DOPA) failed to affect AdoHcy levels. It is concluded that (a) cerebral AdoHcy levels are more tightly regulated than are those of AdoMet after MSO administration, (b) slight fluctuations of AdoHcy levels may be important in regulating AdoHcy hydrolase activity and hence AdoHcy catabolism in vivo, (c) the AdoMet/AdoHcy ratio reflects the absolute AdoMet concentration rather than the transmethylation flux, (d) the decreased AdoMet

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levels in midbrain, cortex, and striatum after MSO with no corresponding decrease in AdoHcy suggest an enhanced AdoMet utilization, hence an increased transmethylation in the MSO preconvulsant state.

INTRODUCTION

Attempts to elucidate the mechanism(s) responsible for the seizure-producing properties of methionine sulfoximine (MSO) have demonstrated that this agent alters methylation processes in brain. Administration of MSO resulted in (a) decreased levels of cerebral *S*-adenosylmethionine (AdoMet) in the rat (1), (b) increased activity of histamine-*N*-methyltransferase (HMT) in rat and mouse brain (2), and (c) increased activity of catechol-*O*-methyltransferase (COMT) in rat brain (2,3). The lack of effect of MSO (in vivo or in vitro) on the activity of the enzyme responsible for AdoMet synthesis (ATP:L-methionine-*S*-adenosyltransferase) (4,5), paired with the finding of the elevated cerebral HMT and COMT activity (in vivo) (2), has led to the suggestion that MSO acts by increasing the flux through methylation pathways (2). The product of these methylations, *S*-adenosylhomocysteine (AdoHcy), has been proposed as a regulator of the activities of HMT (6,7), COMT (8), indolethylamine-*N*-methyltransferase (9), tRNA methyltransferases (10,11), and phenylethanolamine-*N*-methyltransferase (12).

This investigation is concerned with the effect of MSO on AdoHcy levels and on the activity (in vivo) of the enzyme that hydrolyzes AdoHcy to adenosine and homocysteine (AdoHcy hydrolase, E.C. 3.3.1.1.). The effect of methionine on the above parameters was also studied since methionine antagonizes MSO seizures (13,14) as well as the biochemical alterations in AdoMet (1), HMT, and COMT (2). AdoHcy levels after the administration of dihydroxyphenylalanine (DOPA) and cycloleucine were also determined since both agents decrease brain AdoMet , the former by increasing the utilization of methyl groups (15,16), and the latter by inhibiting AdoMet synthesis (16,17). Further, these two drugs, although they markedly decrease AdoMet levels, do not produce seizures. In fact, in the doses we have used, DOPA and cycloleucine injected concurrently with MSO confer some degree of protection against MSO seizures (Schatz and Sellinger, unpublished work).

We have also investigated the validity of using the AdoMet/AdoHcy ratio as an index of transmethylation activity of brain tissue, as has been previously suggested (2,7).

EXPERIMENTAL PROCEDURE

Animals. Adult male Sprague–Dawley rats (120–200 g) and adult male Swiss–Webster mice (25–32 g) were obtained from Spartan Research Animals, Haslett, Michigan.

Materials. S-adenosyl-L-homocysteine (AdoHcy), L-methionine, and L-methionine *d,l*-sulfoximine (MSO) were obtained from Sigma Chemical Co. (St. Louis, Missouri), adenosine from P-L Biochemicals Inc. (Milwaukee, Wisconsin), L-dihydroxyphenylalanine (DOPA), D,L-homocysteine, and L-methionine from ICN Nutritional Biochemicals (Cleveland, Ohio), radioactive adenosine [8-C^{14}] (sp act 52 mCi/mmol), PCS (a tissue solubilizer-scintillant mixture) from Amersham Searle (Arlington Heights, Illinois), silica gel and cellulose TLC plates F-254 (with fluorescent indicator) from EM Laboratories (Elmsford, New York), and Dowex 50W-X8 (200–400 mesh) and disposable glass barrel chromatography columns (0.7 × 10 cm) from Bio-Rad Laboratories (Richmond, California). Cycloleucine (18) was generously supplied by Dr. R. Zand, Biophysics Division, Institute of Science and Technology, University of Michigan.

Drug Treatment and Tissue Preparation. Rats or mice were injected intraperitoneally with saline (10 ml/kg), MSO (0.94 mmol/kg), methionine (4.7 mmol/kg), or a combination of MSO plus methionine, and sacrificed by decapitation. For regional analyses, rat brains were rapidly dissected on ice (19). Whole brains or brain regions were frozen in liquid N_2 and stored at −65°C until analyzed for AdoHcy content.

For measurement of AdoHcy hydrolase activity, whole brains or brain regions were homogenized in 5 vol of 0.05 M potassium phosphate buffer (pH 7.4) and centrifuged at 35,000*g* for 20 min. These supernatants were used as the source of AdoHcy hydrolase.

Analytical. Radioactive AdoHcy was prepared from adenosine [8-C^{14}] and homocysteine using rat liver enzyme purified up to the dialyzed 40–55% ammonium sulfate fraction (20). The enzyme reaction was stopped by addition of 1 N perchloric acid. After neutralization (pH 7) of perchloric acid supernatants with 10 N and 1 N KOH, the samples were centrifuged at 9000*g* for 10 min. The supernatants were lyophilized, dissolved in a small volume of water, streaked on Whatman #3 paper, and run in ethanol–acetic acid–water (65 : 1 : 34) for a distance of 36 cm. The dried chromatograms were inspected under UV light, and the streak containing AdoHcy was cut out and eluted with water and sonication. After successive filtration through GF/A and Millipore 10-μm filters the AdoHcy samples were again lyophilized. After lyophilization the samples were dissolved in water, and the sulfur content (21) and radioactivity of the samples were measured for determination of specific activity. The sulfur content of AdoHcy samples was compared to AdoHcy standards. The sulfur assay is sensitive to 0.01 μmol of AdoHcy. No impurities could be detected in AdoHcy samples chromatographed in ethanol–acetic acid–water (65 : 1 : 34) on silica and Whatman #1 paper, in ethanol–1 M ammonium acetate (70 : 30) on cellulose and silica, and in n-butanol–acetic acid–water (60 : 17 : 25) on paper. The AdoHcy on the chromatograms was identified by UV light, chloroplatinate spray for sulfur compounds, and ninhydrin spray (0.25% in acetone) for amino acids.

AdoHcy levels were measured using a scaled-down isotope dilution method (1,22,24). To frozen brains or brain regions (pooled from 4 rats) were added AdoHcy [8-C^{14}] (sp act 1–7 × 10^5 dpm/μmol) (50 nmol AdoHcy/g tissue) and 1.5–3 ml 1 N perchloric acid. After homogenization, the samples were centrifuged at 9000*g* for 10 min, the supernatants were adjusted to pH 11 using 10 N and 1 N KOH and stirred for 15 min at room temperature to destroy the alkali-labile AdoMet, the pH was readjusted to 7 with 1 N and 0.1 N perchloric acid, after which the samples were centrifuged at 9000*g* for 10 min. These supernatants were stored frozen at −65°C. After thawing, the samples were put on Dowex

50 columns (0.7 × 1.5 or 3 cm) in the H^+ form (23) previously equilibrated with 1 N HCl. The columns were eluted with 2 N HCl until the absorbance at 256 nm was less than 0.02. Elution was continued with water (10 ml) followed by 3 N NH_4OH (24). From 5 ml to 9 ml of the NH_4OH eluate was lyophilized and dissolved in 0.5 ml H_2O, to which was added 2.5 ml sulfur reagent (21) and the absorbance at 500 nm measured. Five milliliters PCS was added to 0.5 ml of sample after the sulfur reaction for counting. Counting efficiency was determined using the channels ratio technique. Recovery of AdoHcy in the NH_4OH eluates was from 50% to 70%. Two standards were carried through the experimental procedure each day. The purity of eluates was monitored as described above. In addition, samples were run bidimensionally [ethanol–acetic acid–water, 65 : 1 : 34 and acetone–water, 80 : 20 (25)] on silica and cellulose F-254 plates and no impurities were detectable. Further, samples of other sulfur-containing compounds (glutathione, L-cysteic acid, cystathionine, MSO, AdoMet, methionine, L-cysteine, L-cystine, D,L-homocysteic acid, and taurine) were carried through the experimental procedure and no interference was detected. This scaled-down isotope-dilution technique in combination with the sensitivity of the sulfur reagent (.01 μmol AdoHcy) allowed measurement of AdoHcy content in 300 mg tissue (assuming an AdoHcy level of 60 nmol/g tissue and 50% loss of AdoHcy during assay).

AdoHcy hydrolase activity was measured in the direction of hydrolysis using radioactive AdoHcy [8-C^{14}] as a substrate and 35,000*g* supernatant as enzyme source (20,26). Studies were conducted using this relatively crude, nondialyzed enzyme preparation since we had previously noted that dialysis reduced the elevation of HMT activity (2) seen after in vivo MSO administration, and we therefore reasoned that dialysis or further purification of AdoHcy hydrolase might remove any effects produced by MSO administration. Kinetic studies using 240-fold purified AdoHcy hydrolase (Schatz, Vunnam, and Sellinger, unpublished results) are at present under way. AdoHcy concentration was 320 μM in all cases. The reaction was linear for the time and enzyme concentrations used. Heated enzyme (90°C for 5 min) was used as blank and incubations were for 15 min and were stopped by heating, after which the tubes were centrifuged (9000*g* for 10 min) to remove any suspended material. The supernatants were lyophilized, dissolved in 75 μl of water, and aliquots were spotted on Whatman #3 paper along with adenosine, inosine, and hypoxanthine and run (18 cm) in ethanol–acetic acid–water (65 : 1 : 34). The R_f values of these compounds are approximately the same in this solvent and clearly separated as one large spot from the substrate (AdoHcy). The chromatograms were dried, the reaction products were visualized by UV light, cut from the chromatograms, added to scintillation vials containing 1 ml H_2O, and sonicated for 10 min, after which 10 ml PCS was added and the vials counted. The amount of product(s) formed was calculated using the specific activity of AdoHcy (1–7 × 10^5 dpm/μmol) and results are reported as nmol AdoHcy hydrolyzed/mg protein/h.

Protein was measured in duplicate by the Lowry procedure (29).

Statistical Analyses. Statistical comparisons were made using the two-tailed Student's *t* test.

RESULTS

Three hours after MSO administration, AdoHcy levels were decreased by 15% in rat brain (Table I) and 27% in mouse brain (Table II).

TABLE I

EFFECT OF METHIONINE OR METHIONINE SULFOXIMINE ON WHOLE RAT BRAIN *S*-ADENOSYLHOMOCYSTEINE LEVELS[a]

Treatment	nmol AdoHcy/g brain	% Change	*P*
Saline	46.3 ± 1.4 (10)	–	–
MSO	39.5 ± 1.4 (10)	−15	<0.01
Methionine	54.1 ± 3.8 (5)	+17	<0.01
MSO + methionine	49.1 ± 0.7 (5)	+6	NS

[a] Rats were injected intraperitoneally with saline (10 ml/kg), MSO (180 mg/kg), methionine (700 mg/kg), or MSO + methionine 3 h prior to sacrifice. AdoHcy values are expressed as means ± SE in (*N*) determinations. Percent change values are as compared to saline treated animals. *P* values were determined by the 2-tailed Student's *t* test. *P* values >0.05 were considered not significant (NS).

Conversely, methionine administration elevated AdoHcy levels in rat (17%) and mouse brain (30%). When both agents were given concurrently, no changes in AdoHcy were observed. Tables I and II also show that AdoHcy levels of mouse and rat brain vary only slightly, those of mouse being 1.5-fold higher.

Lowest AdoHcy values observed were in spinal cord (16.5 nmol/g) (Table III). Regional variation in AdoHcy levels was moderate, ranging from a low of 20 nmol/g in cerebellum to a high of about 60 nmol/g in hypothalamus and midbrain. The effect of MSO on regional AdoHcy levels was quite variable. MSO significantly decreased AdoHcy in brainstem, hypothalamus, and cerebellum (28–38%), moderately decreased AdoHcy in cortex and hippocampus (11% and 17%, respectively), and had no effect in striatum, midbrain, and spinal cord (Table III).

TABLE II

EFFECT OF METHIONINE OR METHIONINE SULFOXIMINE ON WHOLE MOUSE BRAIN *S*-ADENOSYLHOMOCYSTEINE LEVELS[a]

Treatment	nmol AdoHcy/g brain	% Change	*P*
Saline	69.7 ± 1.3 (5)	–	–
MSO	51.1 ± 6.8 (6)	−27	<0.025
Methionine	90.4 ± 6.2 (6)	+30	<0.01
MSO + methionine	74.4 ± 6.3 (5)	+7	NS

[a] Drug treatment, sacrifice time, and expression of results are as in Table I.

TABLE III
EFFECT OF METHIONINE SULFOXIMINE ON *S*-ADENOSYLHOMOCYSTEINE LEVELS IN RAT BRAIN REGIONS AND SPINAL CORD[a]

Region	Treatment	nmol AdoHcy/g brain	% Change	*P*
Cerebellum	Saline	19.9 ± 1.6 (5)	–	–
	MSO	12.4 ± 1.6 (5)	−38	<0.01
Brainstem	Saline	47.6 ± 1.4 (5)	–	–
	MSO	34.4 ± 3.9 (5)	−28	<0.01
Striatum	Saline	48.4 ± 4.2 (4)	–	–
	MSO	44.1 ± 5.4 (4)	−9	NS
Hypothalamus	Saline	58.1 ± 2.4 (4)	–	–
	MSO	40.7 ± 4.1 (4)	−30	<0.005
Midbrain	Saline	59.1 ± 2.5 (4)	–	–
	MSO	62.8 ± 2.7 (4)	+6	NS
Hippocampus	Saline	46.4 ± 7.3 (5)	–	–
	MSO	38.6 ± 5.6 (5)	−17	NS
Cortex	Saline	47.5 ± 3.2 (5)	–	–
	MSO	42.2 ± 3.3 (5)	−11	NS
Spinal cord	Saline	16.5 ± 2.6 (5)	–	–
	MSO	16.4 ± 0.3 (5)	0	NS

[a] MSO treatment, sacrifice time, and expression of results are as in Table I. Regions from the brains of four rats were used for each determination.

AdoHcy hydrolase activity was lowest in hypothalamus, highest in brainstem (28–45 nmol/mg protein/h) (Table IV) and, generally, varied inversely with regional AdoHcy concentration (Table III). MSO treatment markedly decreased AdoHcy hydrolase activity (23–47%) in all regions except the hypothalamus and the spinal cord, where the enzyme activity was slightly increased (Table IV).

Regional rat brain AdoMet/AdoHcy ratios [using AdoMet data from (1) and AdoHcy data from Table III] varied from 0.48 in the hypothalamus to 2.4 in the cerebellum, the next highest being in the striatum (1.02) (Table V). After MSO administration, AdoMet/AdoHcy ratios were decreased in all regions except the hypothalamus. This decrease was greatest in midbrain, cortex, and striatum (43–49%) and less marked in cerebellum, brainstem, and hippocampus (13–24%) (Table V).

Administration of DOPA to mice did not significantly decrease AdoHcy levels, whereas significant decreases in AdoHcy were seen after cycloleucine (16–19%) (Table VI). The reduction of AdoHcy levels was approximately the same whether mice were sacrificed 1 h or 3 h after drug treatment (Table VI).

TABLE IV

EFFECT OF METHIONINE SULFOXIMINE ON THE ACTIVITY OF *S*-ADENOSYLHOMOCYSTEINE HYDROLASE IN RAT BRAIN, BRAIN REGIONS, AND SPINAL CORD[a]

Region	Treatment	AdoHcy hydrolase	% Change	*P*
Cerebellum	Saline	39.5 ± 4.3 (6)		
	MSO	22.6 ± 5.2 (5)	−43	<0.025
Brainstem	Saline	45.2 ± 4.9 (6)		
	MSO	23.8 ± 4.3 (4)	−47	<0.025
Striatum	Saline	36.6 ± 1.7 (5)		
	MSO	26.1 ± 4.3 (5)	−29	<0.05
Hypothalamus	Saline	28.3 ± 8.4 (6)		
	MSO	33.3 ± 11.7 (5)	+18	NS
Midbrain	Saline	32.8 ± 4.9 (5)		
	MSO	24.7 ± 6.2 (5)	−25	NS
Hippocampus	Saline	29.7 ± 2.8 (4)		
	MSO	14.0 ± 4.4 (4)	−53	<0.0125
Cortex	Saline	39.3 ± 4.5 (4)		
	MSO	30.2 ± 3.9 (4)	−23	NS
Whole brain	Saline	36.4 ± 2.8 (6)		
	MSO	26.0 ± 3.2 (5)	−29	<0.025
Spinal cord	Saline	38.1 ± 4.2 (6)		
	MSO	42.7 ± 9.2 (5)	+12	NS

[a] Enzyme activity is expressed as nmol AdoHcy hydrolyzed/mg protein/h and are the means ± SE of (*N*) determinations. For explanation of percent change and *P* value see Table I. Time after MSO or saline, 3 h.

TABLE V

THE EFFECT OF METHIONINE SULFOXIMINE ON *S*-ADENOSYLMETHIONINE/*S*-ADENOSYLHOMOCYSTEINE RATIOS (METHYLATION INDEX) IN RAT BRAIN AND BRAIN REGIONS[a]

Region	Saline	MSO	% Change
Cerebellum	2.40 (48/20)	2.08 (25/12)	−13.5
Brainstem	0.67 (32/48)	0.58 (20/34)	−14.0
Striatum	1.02 (49/48)	0.52 (23/44)	−49.0
Hypothalamus	0.48 (28/58)	0.53 (22/41)	+10.0
Midbrain	0.46 (27/59)	0.26 (17/63)	−43.5
Hippocampus	0.74 (34/46)	0.56 (22/39)	−24.5
Cortex	0.68 (32/47)	0.36 (15/42)	−47.0
Whole brain	0.80 (37/46)	0.51 (20/39)	−36.5

[a] All values are 3-h values with the exception of hypothalamic AdoMet (6 h). AdoMet data is from (1). Numbers in parentheses are AdoMet/AdoHcy values in nmol/g.

TABLE VI
EFFECT OF L-DIHYDROXYPHENYLALANINE OR CYCLOCLEUCINE ON WHOLE MOUSE BRAIN *S*-ADENOSYLHOMOCYSTEINE LEVELS[a]

Treatment	Time (h)	nmol AdoHcy/g brain	% Change	*P*
Saline	1 or 3	72.0 ± 3.1 (8)	–	–
DOPA	1	63.7 ± 5.1 (5)	−12	NS
	3	64.8 ± 5.1 (6)	−10	NS
Cycloleucine	1	58.6 ± 2.5 (6)	−19	<0.005
	3	60.1 ± 0.9 (6)	−16	<0.005

[a] Mice were injected intraperitoneally with saline (10 ml/kg), L-3,4-dihydroxyphenylalanine (200 mg/kg) or cycloleucine (600 mg/kg) 1 or 3 h prior to sacrifice. AdoHcy values are expressed as means ± SE in (*N*) determinations. Since saline injection at 1 or 3 h resulted in no statistically significant effect on AdoHcy levels, 1- and 3-h saline values were pooled. For explanation of percent change and *P* value see Table 1.

DISCUSSION

There is a paucity of information concerning AdoHcy levels in nervous tissue. Rabbit brain AdoHcy content (52 nmol/g) (22) is intermediate between that of rat and mouse brain (Tables I and II). Regional AdoHcy levels have previously not been reported (Table III). Since AdoHcy was found not to have the same regional distribution in rat brain as AdoMet (1) quite different regional AdoMet/AdoHcy ratios were obtained (Table V). Regional AdoHcy values (Table III) did appear, however, to be inversely related to AdoHcy hydrolase activity (Table IV). In mouse brain, which was found to contain significantly higher amounts of AdoHcy than rat or rabbit brain, AdoHcy hydrolase activity was too low to be accurately determined by the methods employed herein. This extreme inverse correlation suggests a prominent role for this enzyme in regulating cellular AdoHcy levels and thus, indirectly, AdoMet-dependent reactions such as those catalyzed by HMT (2) and COMT (8). No such relationship was shown to hold between the concentration of AdoMet and the activities of HMT (1) and COMT (30).

The increase in cerebral AdoHcy levels after methionine noted in both the mouse and the rat (Tables I and II) has also been reported in rabbit brain (22). Conversely, the administration of MSO resulted in decreased AdoHcy levels in rat and mouse brain, this decrease being prevented by the concurrent administration of methionine (Tables I and II). The increased AdoHcy after methionine and decreased AdoHcy

after MSO, and cycloleucine (Table VI), although similar to the same changes noted for AdoMet (1,15,16), were much less marked, indicating that cellular AdoHcy levels are under a more tightly regulated control than are those of AdoMet.

Since both MSO and DOPA have been proposed as causing an increased transmethylation flux (1,2,15), it was expected that, as AdoMet utilization accelerated, AdoHcy levels would increase rather than decrease. The observed decrease of AdoHcy after MSO may receive a tentative explanation by involing kinetic considerations relating to the affinity of brain AdoHcy hydrolase for AdoHcy. The reported apparent K_m values for AdoHcy of AdoHcy hydrolase from a number of sources are as follows: 41 μM, spinach beet (27); 33 μM, rat small intestine (28); 30 μM, rat liver (28); and about 40 μM, rat brain supernatant, and 240-fold purified AdoHcy hydrolase from the latter source (Schatz, Vunnam, and Sellinger, unpublished results). Since the cerebral AdoHcy levels were found to be in the same molar concentration range (Tables I and III) as the apparent K_m values for AdoHcy, the reduction of AdoHcy hydrolase activity seen after MSO (Table IV) is a direct reflection of the close dependence of the AdoHcy hydrolase activity on the endogenous AdoHcy levels. In view of the fact that the enzyme also acts in reverse, i.e., it synthesizes AdoHcy, and that it is highly sensitive to both end products of the synthetic and the hydrolytic reactions (20,26,27), further studies are necessary to fully ascertain the importance of AdoHcy hydrolase in the regulation of AdoHcy metabolism and thereby in the more general context of its role in the control of transmethylation in brain tissue.

Since AdoHcy levels appeared more resistant to changes by drug treatment than were the levels of AdoMet (1,15,16), the applicability of the AdoMet/AdoHcy ratio as a cellular methylation index (1,7) seems doubtful. Rather, this ratio is a reflection of the absolute levels of AdoMet which are per se indicative of the state of flux of cellular transmethylation reactions. The situation encountered in the striatum, the cortex, and the midbrain (Table V) thus becomes a real measure of an enhanced AdoMet utilization, since in these regions AdoMet levels were decreased by MSO (1) while those of AdoHcy remained unchanged.

Although cycloleucine and DOPA also deplete cerebral AdoMet they do so by different mechanisms. Cycloleucine effectively decreases methylation (16) because it inhibits AdoMet synthesis (4,17) and may also inhibit AdoHcy formation (Table VI). DOPA, on the other hand, competes with other methyl acceptors for AdoMet and consequently reduces the methylations of histamine (7,32) and norepinephrine (32,33).

These and presumably other as yet unidentified effects of DOPA and cycloleucine on cellular methylation could account for the partial protective effect these substances exerted against MSO. An alternative explanation of this protective effect may be that DOPA and cycloleucine, like methionine (34,35), compete with MSO for uptake into cerebral tissue. DOPA has been shown to compete with methionine for uptake into synaptosomes (36), and, speculatively, this may hold true for cycloleucine as well. Some support for this notion was presented by Zand et al. (18), who noted that the intraperitoneal administration of a number of cyclic and bicyclic amino acids results in a marked reduction of cerebral methionine and, in two cases, in marked increases of brain cysteine. Finally, the possibility exists that the MSO-induced regional deficits in either AdoMet or AdoHcy may underlie regional deficiencies of methionine and/or homocysteine. Although for methionine we have previously shown that soon after MSO administration there is a significant elevation of its cerebral levels which progressively return to normal values within 4 h (37), no data are available for homocysteine, the cerebral levels of which are to date unknown.

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