Journal of Neurochemistry
Raven Press, New York

0022-3042/83/0301-0836/$03.00/0

Biosynthesis of Polyamines in Mouse Brain: Effects of Methionine Sulfoximine and Adenosylhomocysteine

Raffaele Porta, Robert A. Schatz, Stephen B. Tatter, and Otto Z. Sellinger

Laboratory of Neurochemistry, Mental Health Research Institute, University of Michigan Medical Center, Ann Arbor, Michigan, U.S.A.

Abstract: This study examines the consequences on cerebral polyamine biosynthesis of increases and decreases in cerebral methylation. Increases were elicited by administering the convulsant agent methionine sulfoximine (MSO) and decreases by elevating *in vivo* the cerebral levels of the methylation inhibitor *S*-adenosylhomocysteine. Following the intraventricular (i.vt.) administration of one of the two possible polyamine precursors, [1,4-^{14}C]putrescine, the specific radioactivity (sra) of the newly formed [^{14}C]spermidine remained unchanged. Conversely, after i.vt. L-[3,4-^{14}C]methionine, the other polyamine precursor, significantly higher sra values for [^{14}C]spermidine and [^{14}C]spermine were recorded in the brains of the MSO-treated animals. [^{14}C]*S*-adenosylmethionine in the brain of the MSO-treated animals was also more highly labeled following [1-^{14}C]-methionine, indicating its accelerated formation relative to controls. We also investigated the effect of the administration of adenosine + homocysteine, a treatment that results in elevated brain adenosylhomocysteine levels, on polyamine biosynthesis from [3,4-^{14}C]-methionine. The results of these experiments show both significantly lower sra values for [^{14}C]spermidine and [^{14}C]spermine and significantly higher than control endogenous methionine levels, a clear sign of the existence of a retardation in the conversion of methionine to polyamines under these conditions. In conclusion, the present study demonstrates that while interference with cerebral methylation results in significant alterations of the rate of formation of the methionine moiety of spermidine and spermine, it has no effect on the entry of the putrescine moiety into the two polyamine molecules. **Key Words:** Polyamines—Biosynthesis—Methionine sulfoximine—*S*-adenosylhomocysteine. **Porta R. et al.** The biosynthesis of polyamines in mouse brain: Effects of methionine sulfoximine and adenosylhomocysteine. *J. Neurochem.* **40**, 836–841 (1983).

Previous research has demonstrated that the administration of the convulsant agent L-methionine-*dl*-sulfoximine (MSO) results in an accelerated transfer of *S*-adenosyl-L-methionine (AdoMet)-derived methyl groups to a number of cerebral methyl acceptor molecules, which, to date, include histamine, transfer ribonucleic acids, proteins, and phospholipids (Schatz and Sellinger, 1975*a*; Salas et al., 1977; Schatz et al., 1978; 1982*a,b*,; Dainat et al., 1978; Sellinger and Schatz, 1979). Unexpectedly, the levels of the resulting *S*-adenosyl-L-homocysteine (AdoHcy), a potent inhibitor of all methylations, were not found to be correspondingly elevated, but rather somewhat decreased (Schatz et al., 1977; 1982*a,b*), possibly because of the continued, effective AdoHcy-removal action of cerebral AdoHcy hydrolase (Schatz et al., 1979), despite its moderate inhibition by MSO (Schatz et al., 1977).

In conjunction with our interest in understanding the mechanism whereby MSO "stimulates," albeit indirectly, cerebral methylations (Sellinger and Schatz, 1979), we recently investigated means of counteracting the effects of this convulsant agent by retarding cerebral methylations. An effective way to do this was found to be the administration of adenosine and homocysteine thiolactone, jointly and upwards of a dose of 200 mg/kg of each sub-

Received July 30, 1982; accepted September 21, 1982.
Address correspondence and reprint requests to Otto Z. Sellinger, Laboratory of Neurochemistry, Mental Health Research Institute, University of Michigan Medical Center, Ann Arbor, MI 48109, U.S.A.

The present address of Raffaele Porta is Department of Biochemistry, 1st Medical School, University of Naples, via Costantinopoli 16, 80138 Naples, Italy.

The present address of Robert A. Schatz is Department of Toxicology, College of Pharmacy, Northeastern University, 360 Huntington Avenue, Boston, Massachusetts.

The present address of Stephen B. Tatter is The Rockefeller University, 1230 York Avenue, New York, New York.

Abbreviations used: AdoHcy, *S*-Adenosylhomocysteine; AdoMet, *S*-Adenosylmethionine; i.vt., Intraventricular; MSO, L-Methionine-*d,l*-sulfoximine; sra, Specific radioactivity.

stance. As clearly shown by Schatz et al. (1981*a*), at a dose of 500 mg/kg this treatment causes a sevenfold elevation of cerebral AdoHcy levels, a condition that effectively annihilates any MSO-elicited stimulation of the methyl transfer reactions enumerated above (Schatz et al., 1982*b*). Thus, two different ways of modulating cerebral methylations have become available, an upward modulation in response to MSO and a downward one in response to AdoHcy.

In addition to being the universal methyl donor molecule, AdoMet is also a precursor of the cerebral polyamines spermidine and spermine via its decarboxylation and the subsequent coupling of the *n*-propylamino moiety of decarboxylated AdoMet with putrescine and spermidine. Since polyamines have been found to possess excitatory properties when introduced directly into the brain of rodents (Anderson et al., 1975), and since a relationship between audiogenic seizure sensitivity and brain polyamines has recently been established in deermice (Porta et al., 1981*a*), it became of interest to investigate the possibility that treatments that affect cerebral methylations, and hence the utilization and disposition of AdoMet, also disrupt the synthetic mechanisms leading to spermidine and spermine. We decided, therefore, to examine the effects of MSO and of high brain AdoHcy levels on the respective incorporation of putrescine and methionine into cerebral spermidine and spermine. A partial account of some of the findings has already appeared (Porta et al., 1981*b*; 1982).

MATERIALS AND METHODS

A model 204 liquid chromatograph with models 440 uv absorbance detector (254 nm), 6000A solvent delivery system, and U6K universal injector (all from Waters Associates Inc., Milford, MA) was used for high performance liquid chromatography (HPLC) analyses. The photometer output was displayed on an Omniscribe strip chart recorder (Texas Instruments, Austin, TX). The column used for reverse-phase HPLC was μBondapak C_{18} (4 mm i.d. × 30 cm) (Waters Associates).

[1,4-^{14}C]Putrescine dihydrochloride (122 mCi/mmol), [^{14}C]spermidine trihydrochloride (122 mCi/mmol), [^{14}C]spermine tetrahydrochloride (122 mCi/mmol), and L-[1-^{14}C]methionine (59 mCi/mmol) were obtained from Amersham-Searle (Arlington Heights, IL); L-[3,4-^{14}C]methionine (49 mCi/mmol) from Research Products International Corp. (Elk Grove Village, IL); [^{14}C]adenosylmethionine (57 mCi/mmol) from New England Nuclear (Boston); putrescine dihydrochloride, spermidine trihydrochloride, spermine tetrahydrochloride, L-methionine-*d,l*-sulfoximine, adenosine, and D,L-homocysteine thiolactone-HCl from Sigma Chemical Co. (St. Louis). *S*-Adenosylmethionine disulfate di-*p*-toluenesulfonate was generously donated by Dr. G. Stramentinoli (BioResearch Laboratories, Liscate, Italy). Dowex 50 W-X8 (200–400 mesh) was from BioRad (Richmond, CA). All other reagents were analytical grade.

Adult male Swiss-Webster mice (25–30 g) were from Charles River Laboratories (Portage, MI) and were housed (7:00 a.m. to 7:00 p.m. light cycle followed by a 12-h dark cycle) in stainless steel cages (six mice/cage) for at least 48 h before use. The mice were fasted overnight but were allowed water *ad libitum* prior to the experimental procedures. After treatment each animal was transferred to an individual cage.

Injection schedules and routes of administration

All injections were carried out between 8:00 and 11:00 a.m. MSO was given intraperitoneally (i.p.) at a dose of 150 mg/kg in saline (10 ml/kg). The mixture of adenosine + homocysteine thiolactone (dissolved in saline/Tween 80, 20 ml/kg) was injected i.p. at doses of 200 or 500 mg/kg, 40 min prior to death. [1,4-^{14}C]Putrescine (0.25 μCi/mouse), L-[3,4-^{14}C]methionine (1 or 2 μCi/mouse), and L-[1-^{14}C]-methionine (2 μCi/mouse) were injected into the lateral cerebral ventricle in a volume of 10 μl of artificial cerebrospinal fluid (Merlis solution) (Schatz et al., 1978). For intraventricular (i.vt.) injections the animals were lightly anesthetized with ether and a small piece of scalp was removed for ease in identification of the injection site. They were killed by immersion (head first) into liquid nitrogen and were stored frozen overnight at −20°C. Whole brains were rapidly removed, immediately refrozen in liquid nitrogen, and kept at −20°C until the time of assay.

Determination of the levels and specific radioactivities of methionine, *S*-adenosylmethionine, and polyamines

The quantitative determinations, using HPLC procedures, of tissue polyamine and AdoMet levels were carried out as previously described (Porta et al., 1981*a*). In experiments in which [1,4-^{14}C]-putrescine, L-[3,4-^{14}C]-methionine, or L-[1-^{14}C]-methionine was injected, we determined the amounts of radioactivity in the newly produced [^{14}C]polyamines or in [^{14}C]AdoMet by collecting 1.0-ml fractions from the chromatographic runs. After addition of 6 ml of ACS (a tissue solubilizer-scintillation mixture, Amersham-Searle, Arlington Heights, IL), the fractions were counted and their radioactivity was used to calculate the specific radioactivity (sra, dpm/nmol) values. Tissue L-methionine levels were determined by the procedure of Parrilla et al. (1973), with some modifications (Porta et al., 1981*a*). The determination of the cerebral methionine sra, following L-[1-^{14}C]methionine i.vt. injections, was performed as previously described (Porta et al., 1981*a*).

Statistical analysis

The statistical significance was evaluated by Student's two-tailed *t*-test at a 0.05 level of significance.

RESULTS

Biosynthesis of brain polyamines in MSO-treated mice

The determination of the steady state levels of brain polyamines was performed 30, 90, 135, and 180 min after administration of MSO. A significant increase (~40%) of spermidine and spermine levels was observed only at 180 min, a time when the con-

vulsant action of MSO can no longer be effectively reversed (Sellinger et al., 1968). The levels of brain putrescine remained unchanged throughout the 3-h post-MSO period.

Since in order for the methionine carbon to enter the spermidine moiety the injected methionine must first mix with the endogenous pool of methionine and then react with ATP and ATP:methionine adenosyltransferase to form the polyamine precursor AdoMet, it was important to determine the levels of brain methionine and AdoMet 3 h after MSO administration. The sra values of [^{14}C]methionine and [^{14}C]AdoMet were also determined. Table 1 shows significantly lower methionine and AdoMet contents in the MSO-treated brains. Conversely, while the sra values of [^{14}C]methionine were significantly higher than control at both pulse times, those of [^{14}C]AdoMet appeared higher only at 1 h.

To analyze the precursor flux into spermidine and spermine in the MSO-treated animals, L-[3,4-^{14}C]methionine or [1,4-^{14}C]putrescine was injected i.vt. in separate experiments and the animals killed 1 or 3 h later. As shown in Table 2, the sra values of [^{14}C]spermidine and [^{14}C]spermine following methionine were significantly higher at 1, but not at 3 h. Following [^{14}C]putrescine, on the other hand, there was no difference between the [^{14}C]spermidine sra values of the MSO-treated and the control animals at either 1 or 3 h. Moreover, the [^{14}C]spermine fraction never had any radioactivity (Table 3).

Formation of [^{14}C]spermidine and [^{14}C]spermine after *in vivo* elevation of brain AdoHcy levels

It was previously reported (Schatz et al., 1981*a*) that the coadministration of adenosine and homocysteine thiolactone results in an elevation of brain AdoHcy levels. Since such high levels of brain AdoHcy cause cerebral methylation to be inhibited (Schatz et al., 1981*a*,*b*), it was quite reasonable that the effects of such a state on polyamine biosynthesis be investigated. The results (Table 4) show a significant reduction relative to controls of the sra values of [^{14}C]spermidine and [^{14}C]spermine formed in 1 h after an i.vt. pulse of [3,4-^{14}C]methionine. In contrast to the results after MSO (Table 3), the levels of brain methionine became significantly elevated (55%) after 500 mg/kg of adenosine + homocysteine thiolactone, whereas, in confirmation of our previous results (Schatz et al., 1981*a*,*b*), the levels of brain AdoMet failed to change.

DISCUSSION

In the neurochemical literature there is little information specifically concerning the formation of polyamines following the separate administration of labeled methionine versus putrescine. Antrup and Seiler (1980) described long-term experiments designed to determine the turnover rates of polyamines following repeated daily i.p. doses of [^{14}C]methionine and [^{14}C]putrescine. These workers calculated apparent biological half-lives ($t_{1/2}$) of 13–16 days for spermidine and of 18 days for spermine following methionine, whereas after putrescine these $t_{1/2}$ values became much longer—42 days. Since this discrepancy in half-lives, as determined from the two precursors, held only for brain tissue, the authors concluded that this particular feature "indicates an exceptional characteristic of polyamine metabolism in brain." The turnover of polyamines in mouse brain, using [^{14}C]putrescine administered i.vt., was also examined by Hietala et al. (1980), who determined the sra values for [^{14}C]spermidine and [^{14}C]spermine over a 60-h period, noting the attainment of maximum values at about 20 h. The decline in the sra values of [^{14}C]-putrescine itself following its i.vt. administration was determined as well and found to be extremely rapid (Pajunen et al., 1980), yielding a half-life of about 12 h.

Given the demonstrated feasibility of utilizing i.vt.-administered precursors of the polyamines to effect the latter's labeling in brain tissue, it appeared of interest to generate conditions that would differentially influence the disposition of putrescine and methionine into spermidine and spermine. This possibility seemed even more attractive given the

TABLE 1. *Effect of MSO on the endogenous levels and the specific radioactivities of mouse brain [^{14}C]methionine and [^{14}C]S-adenosylmethionine*

Pulse time (h)	[^{14}C]Methionine			[^{14}C]*S*-Adenosylmethionine		
	Control	MSO	% Change	Control	MSO	% Change
A						
1	0.85 ± 0.08 (6)	1.86 ± 0.23 (7)[b]	+218	4.56 ± 0.41 (6)	6.52 ± 0.56 (7)[a]	+43
3	0.14 ± 0.02 (8)	0.28 ± 0.02 (8)[c]	+100	1.60 ± 0.16 (8)	1.80 ± 0.18 (8)	+11.3
B[d]	144.8 ± 10.1 (6)	111.4 ± 8.7 (7)[a]	−23	22.9 ± 1.91 (6)	12.4 ± 1.20 (7)[a]	−45.5

Mice were given an i.p. injection of saline or MSO (150 mg/kg) 3 h before killing. L-[1-^{14}C]methionine: 2 and 1 μCi/10 μl were injected for the determination of the specific radioactivities of *S*-adenosylmethionine and methionine, respectively. Values are expressed as means ± SEM. A: dpm/nmol; B: nmol/g. Numbers in parentheses represent the number of animals in each group.

[a–c] Significantly different from control value: *a*, $p < 0.02$; *b*, $p < 0.005$; *c*, $p < 0.001$.

[d] Determined 3 h after MSO.

TABLE 2. *Effect of MSO on the conversion of [3,4-^{14}C]methionine to [^{14}C]spermidine and [^{14}C]spermine in mouse brain*

Pulse time (h)	[^{14}C]Spermidine			[^{14}C]Spermine		
	Control	MSO	% Change	Control	MSO	% Change
1[c]	28.4 ± 1.0 (7)	42.7 ± 3.9 (11)[a]	+50.0	40.1 ± 1.2 (7)	60.6 ± 4.3 (11)[b]	+50.4
3[d]	22.4 ± 1.6 (6)	25.4 ± 0.08 (12)	+11.5	38.8 ± 2.4 (6)	40.6 ± 1.1 (8)	+10.4

Mice were given an i.p. injection of MSO (150 mg/kg) 3 h before killing. Numbers in parentheses represent the number of animals in each experimental group. Values are in dpm/nmol.

[a] Significantly different from control value: $p < 0.005$.

[b] Significantly different from control value: $p < 0.001$.

[c] 2 μCi of [3,4-^{14}C]methionine was injected.

[d] 1 μCi of [3,4-^{14}C]methionine was injected.

previously documented finding (Antrup and Seiler, 1980) that "the two parts of the polyamine structure deriving from ornithine and methionine, respectively, can have widely different biological life spans." The experimental conditions chosen in the present study to create the desired circumstances were those apt to change the state of cerebral methylation. These were established by accelerating the rate of methyl transfer, i.e., administering methionine sulfoximine, and by creating an acute methylation-inhibitory state by the administration of adenosine + homocysteine thiolactone.

The administration of MSO caused significant, but moderate, reductions in the endogenous levels of brain methionine and AdoMet, 23 and 45%, respectively (Table 1). Yet, the sra values of [^{14}C]methionine in the MSO-treated brains were higher than control by as much as 218 and 100% at, respectively, 1 and 3 h after MSO. These results indicate a preferential effect of MSO on the entry and maintenance of exogenous [^{14}C]methionine in the cellular pool, as against its effect on the efflux or the rate of utilization of endogenous methionine. The effect of MSO at the level of brain AdoMet was somewhat different, since the size of the reduction of its endogenous content (as percentage), noted several times previously (Schatz et al., 1975*b*; 1982*a*,*b*), was virtually equal to the percentage increase of its sra. Still, the MSO-stimulated flux of [^{14}C]-methionine into the cellular pool containing [^{14}C]AdoMet was readily reflected by the significantly increased sra values of [^{14}C]spermidine and [^{14}C]spermine in the brains of the MSO-treated mice (Table 2), relative to controls. This was particularly evident at 1 h.

The data shown in Table 3 support the conclusion of Antrup and Seiler (1980) regarding differences in the rates of entry of methionine and putrescine into the spermidine and spermine structures, inasmuch as they emphasize the absence of an effect of a methylation-activating agent, MSO, on the moiety of the polyamines not derived from AdoMet. Thus, our findings strengthen the argument favoring entirely independent fluxes of putrescine and of AdoMet toward polyamines. Moreover, in partial accord with the findings of Hietala et al. (1980), we confirmed the fact that formation of spermine from putrescine is markedly slower—in our case nil (Table 3)—than that of spermidine. After about 5 h of i.vt. putrescine administration Hietala et al. noted a spermine/spermidine ratio of about 0.2. Similarly, the threefold decline in the sra of [^{14}C]putrescine from 1 to 3 h (Table 3) is in accord with the data of Pajunen et al. (1980), who noted a similar, albeit somewhat slower, decline of the sra of [^{14}C]putrescine during the first 5 h following its injection.

The downward modulation of cerebral methylation rates can be effectively achieved and maintained by increasing brain levels of AdoHcy. Such an increase occurs within minutes of the i.p. administration of 200 mg/kg of adenosine and homocysteine thiolactone (Schatz et al., 1981*a*). It has been shown conclusively that decreased transmethylation rates are an immediate consequence of the resulting high AdoHcy brain levels (Schatz et

TABLE 3. *Effect of MSO on the conversion of [1,4-^{14}C]putrescine to [^{14}C]spermidine in mouse brain*

Pulse time (h)	[^{14}C]Putrescine		[^{14}C]Spermidine	
	Control	MSO	Control	MSO
1	64.7 ± 4.8 (6)	57.0 ± 3.6 (6)[a]	547.5 ± 47.5 (6)	514.8 ± 48.5 (6)
3	23.5 ± 1.7 (11)	24.0 ± 0.7 (15)[a]	507.0 ± 21.0 (10)	554.4 ± 25.0 (14)

Mice were given an i.p. injection of MSO (150 mg/kg) 3 h before killing. Values are in dpm/nmol and are expressed as means ± SEM. Numbers in parentheses represent the number of animals in each group.

[a] p values were greater than 0.05.

TABLE 4. *Effect of adenosine + homocysteine thiolactone on the conversion of L-[3,4-^{14}C]methionine to [^{14}C]spermidine and [^{14}C]spermine in mouse brain*

Treatment[a]	Spermidine			Spermine		
	dpm/nmol	% Change[b]	p[c]	dpm/nmol	% Change	p[c]
Vehicle	12.32 ± 0.69	—	—	16.76 ± 0.94	—	—
200 mg/kg	9.42 ± 1.24	−23.5	NS	14.62 ± 1.73	−12.8	NS
500 mg/kg	8.84 ± 0.67	−28.2	<0.005	13.00 ± 1.19	−22.4	<0.05

NS: Not significant.

[a] Adenosine + homocysteine thiolactone were administered i.p. 40 min before killing; L-[3,4-^{14}C]-methionine (1.0 μCi) was injected i.vt. 1 h before killing. Six animals were used in each group. Values are means ± SEM.

[b] Compared with vehicle-treated mice.

[c] Determined by Student's *t*-test (two-tailed).

al., 1981*a,b*), since all methyl transfer reactions tested to date were found to be markedly decreased. These have included the formation of *N*-methylhistamine from histamine (Schatz et al., 1981*a*), the carboxylmethylation of cerebral proteins (Schatz et al., 1981*b*), and the formation of cerebral phosphatidyl-*N,N*-dimethylethanolamine and lecithin (Schatz et al., 1981b). In additional experiments, designed to test the existence of an obverse relationship between the action of MSO and high brain AdoHcy levels, we have demonstrated that a timely adenosine + homocysteine thiolactone treatment (Schatz et al., 1981*c;* 1982*a,b,c*) causes a reversal of all MSO-elicited stimulations of cerebral methylation. Thus, it was not unexpected to find a decreased rate of [3,4-^{14}C]methionine incorporation into the spermidine and spermine structures following the administration of 200 mg/kg of adenosine and homocysteine thiolactone (Table 4).

Taken together with the stimulatory effect of MSO on [^{14}C]spermidine synthesis from [^{14}C]methionine (Table 2), the present findings clearly show that it is possible to modulate upward or downward the incorporation of methionine into the n-propylamino moiety of brain polyamines by suitable adjustments in the state of cerebral methylation. Similarly, in nonneural systems, *in vivo* alterations in polyamine concentrations regulate AdoMet-dependent transmethylation reactions (Mach et al., 1982), whereas the administration of AdoMet leads to changes in polyamine levels (Ientile et al., 1981).

A particularly interesting aspect of the present study was its possible relevance to the question of the mechanism of MSO seizures. We reasoned that, by virtue of the capacity of MSO to stimulate the formation of AdoMet (Schatz et al., 1982*b*), its utilization for purposes of methyl transfer reactions (Salas et al., 1977; Dainat et al., 1978; Schatz et al., 1975*a;* 1978; 1981*c;* 1982*b*), and the overall rate at which [^{14}C]methionine enters the polyamine structures (this study), the drug should also cause elevations in brain spermidine and spermine, compounds with known convulsant properties (Anderson et al., 1975; Shaw, 1979; Seiler, 1981). The significantly increased brain levels of the two polyamines 3 h after MSO (see Results) seem to support the validity of this notion. Furthermore, we also show a decreased conversion of [^{14}C]methionine into [^{14}C]spermidine and [^{14}C]spermine when brain AdoHcy levels are elevated and when, as Schatz et al. (1982*b,c*) have shown, MSO seizures are effectively antagonized.

Acknowledgment: This work was supported by a grant from the U.S. Public Health Service, NINCDS 06294 (OZS).

REFERENCES

Anderson D. J., Crossland J., and Shaw G. G. (1975) The actions of spermidine and spermine on the central nervous system. *Neuropharmacology* **14**, 571–577.

Antrup H. and Seiler N. (1980) On the turnover of polyamines spermidine and spermine in mouse brain and other organs. *Neurochem. Res.* **5**, 123–143.

Dainat J., Salas C. E., and Sellinger O. Z. (1978) Alterations of the specificity of brain tRNA methyltransferase and of the pattern of brain tRNA methylation *in vivo* by methionine sulfoximine. *Biochem. Pharmacol.* **27**, 2655–2658.

Hietala O. A., Pajunen A. E. I., Lapinjoki S. P., and Piha R. S. (1980) Interconversion of polyamines in mouse brain. *Acta Univ. Oulu.* A97, [*Biochem.*] **29**, 49–54.

Ientile P., Macaione S., deLuca G., Rotiroti D., and Di Giorgio R. M. (1981) Effects of SAM on polyamine levels and on ODC and GAD activities in rat retina during early postnatal development. *Bull. Mol. Biol. Med.* **6**, 81–92.

Mach M., Kersten H., and Kersten W. (1982) Regulation of tRNA methyltransferase activities by spermidine and putrescine. *Biochem. J.* **202**, 153–162.

Pajunen A. E. I., Lapinjoki S. P., Hietala O. A., and Piha R. S. (1980) Polyamine turnover in mouse brain. *Acta Univ. Oulu.* A97, [*Biochem.*] **29**, 39–47.

Parrilla R., Ayuso-Parrilla M. S., and Goodman M. N. (1973) Determination of some L-amino acids in biological samples by aminoacylation of tRNA. *Anal. Biochem.* **54**, 362–369.

Porta R., Doyle R. L., Tatter S. B., Wilens T. E., Schatz R. A., and Sellinger O. Z. (1981*a*) The biosynthesis of polyamines in the brain of audiogenic seizure-susceptible and -resistant deermice. *J. Neurochem.* **37**, 723–729.

Porta R., Schatz R. A., and Sellinger O. Z. (1981*b*) The modulation of brain methylation affects brain polyamine biosynthesis. *Trans. Am. Soc. Neurochem.* **12**, 150.

Porta R., Schatz R. A., and Sellinger O. Z. (1982) Alterations in

methylation affect polyamine biosynthesis in mouse brain, in *The Biochemistry of S-Adenosylmethionine and Related Compounds* (Borchardt R. T., Usdin E., and Creveling C. R., eds), pp. 581–588. Macmillan, London.

Salas C. E., Ohlsson W. G., and Sellinger O. Z. (1977) The stimulation of cerebral N^2-methyl and N_2^2-dimethyl guanine-specific tRNA methyltransferases by methionine sulfoximine: An *in vivo* study. *Biochem. Biophys. Res. Commun.* **76**, 1107–1115.

Schatz R. A. and Sellinger O. Z. (1975*a*) The elevation of cerebral histamine-*N*- and catechol-*O*-methyltransferase activities by L-methionine-*dl*-sulfoximine. *J. Neurochem.* **25**, 73–78.

Schatz R. A. and Sellinger O. Z. (1975*b*) Effect of methionine and methionine sulfoximine on rat brain S-adenosylmethionine levels. *J. Neurochem.* **24**, 63–66.

Schatz R. A., Vunnam C. R., and Sellinger, O. Z. (1977) *S*-Adenosyl-L-homocysteine in brain: Regional concentrations, catabolism and the effects of methionine sulfoximine. *Neurochem. Res.* **2**, 27–38.

Schatz R. A., Frye K., and Sellinger O. Z. (1978) Increased *in vivo* methylation of [^{3}H]-histamine in the methionine sulfoximine epileptogenic mouse brain. *J. Pharmacol. Exp. Ther.* **207**, 794–800.

Schatz R. A., Vunnam C. R., and Sellinger O. Z. (1979) *S*-Adenosyl-L-homocysteine hydrolase from rat brain: Purification and some properties, in *Transmethylation* (Usdin E., Borchardt R. T., and Creveling C., eds), pp. 143–153. Elsevier/North Holland Press, New York.

Schatz R. A., Wilens T. E., and Sellinger O. Z. (1981*a*) Decreased transmethylation of biogenic amines after *in vivo* elevation of brain *S*-adenosylhomocysteine. *J. Neurochem.* **36**, 1739–1748.

Schatz R. A., Wilens T. E., and Sellinger O. Z. (1981*b*) Decreased *in vivo* protein and phospholipid methylation after *in vivo* elevation of brain *S*-adenosylhomocysteine. *Biochem. Biophys. Res. Commun.* **98**, 1097–1107.

Schatz R. A., Wilens T. E., and Sellinger O. Z. (1981*c*) The elevation of brain *S*-adenosylhomocysteine *in vivo* counteracts the MSO-induced increase in phospholipid and protein carboxymethylation. *Trans. Am. Soc. Neurochem.* **12**, 151.

Schatz R. A., Wilens T. E., Tatter S. B., and Sellinger O. Z. (1982*a*) Hypermethylation in the MSO-epileptogenic brain: reversal by dilantin and phenobarbital, in *The Biochemistry of S-Adenosylmethionine and Related Compounds*. (Borchardt R. T., Usdin E., and Creveling C., eds), pp. 675–679. Macmillan, London.

Schatz R. A., Wilens T. E., Tatter S. B., and Sellinger O. Z. (1982*b*) The elevation of brain *S*-adenosylhomocysteine counteracts seizures and brain hypermethylation induced by methionine sulfoximine. Submitted for publication.

Schatz R. A., Wilens T. E., Tatter S. B., and Sellinger O. Z. (1982*c*) Hypermethylation in the MSO-epileptogenic brain: Reversal by dilantin, phenobarbital or adenosine plus homocysteine. *Toxicologist* **2**, 2.

Seiler N. (1981) Polyamine metabolism and function in brain. *Neurochem. Int.* **3**, 95–110.

Sellinger O. Z. and Schatz R. A. (1979) Cerebral utilization of adenosylmethionine and adenosylhomocysteine: Effects of methionine sulfoximine, in *Biochemical and Pharmacological roles of Adenosylmethionine and the Central Nervous System.* (Zappia V., Usdin E., and Salvatore F., eds), pp. 89–103. Pergamon Press, Elmsford.

Sellinger O. Z., Azcurra J. M., and Ohlsson W. G. (1968) Methionine sulfoximine seizures. VIII. The dissociation of the convulsant and glutamine synthetase inhibitory effects. *J. Pharmacol. Exp. Ther.* **164**, 212–222.

Shaw G. G. (1979) The polyamines in the central nervous system. *Biochem. Pharmacol.* **28**, 1–6.