Neurochemical Research, Vol. 8, No. 12, 1983

THE EFFECT OF S-ADENOSYL-L-METHIONINE ON ISCHEMIA-INDUCED DISTURBANCES OF BRAIN PHOSPHOLIPID IN THE GERBIL

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Accepted June 1, 1983

Brain ischemia was produced in gerbils (*Meriones unguiculatus*) by the bilateral ligation of the carotid arteries with reported procedures. Changes in the energy status of brain demonstrated that carotid ligation was effective. At different time intervals from ligation, groups of gerbils were given either saline or S-Adenosyl-L-methionine (SAMe) by the intraventricular (i.v.) route (1.6 mg/Kg body wt. twice, at each 10 min interval), or by the intraperitoneal (i.p.) administration (200 mg/Kg body wt.) or subcutaneously (s.c.) with 40 mg/Kg body wt, daily, for two weeks. Control animals, with and without SAMe, together with the ischemic groups, were decapitated directly into liquid nitrogen, 10 min after ligation. Brain neutral and polar lipid, together with free fatty acids, which were all labeled in vivo by the intraventricular injection of [1-^{14}C]arachidonic acid 2 hr prior to ligation, were extracted, purified and separated by conventional procedures. SAMe when injected i.v. or i.p. noticeably corrected the changes in polar lipid by reversing the decrease of brain phosphatidylcholine and choline plasmalogen, as well as of their labeling, which was due to ischemia. Concurrently with this action,

This work was carried out by a research grant from the Consiglio Nazionale delle Ricerche (Rome, Italy) through the contribute CT82.02138.04/115.

0364-3190/83/1200-1597$03.00/0

SAMe treatment (i.v. and i.p.) also provided to some extent to re-establish the normal level of labeling of ethanolamine lipids. When SAMe was given s.c., no effect was present. SAMe had no effect on the increase of free fatty acid and diglyceride due to ischemia. The prevention by SAMe of the changes of choline lipids suggests that a stimulation of the methyltransferase reaction may occur in the ischemic brain, due to increased substrate (SAMe) availability. This effect may be important for cell survival, since membrane phospholipid derangements alter the properties of the membrane.

INTRODUCTION

S-Adenosyl-L-methionine (SAMe) is known to play an important role in several metabolic pathways, including phospholipid methylation (1). Endogenous SAMe distribution in several mammalian tissues has been reported by Baldessarini and Kopin (2). The effectiveness of SAMe administration to increase its levels in tissue, and particularly those of brain (3, 4), has prompted a series of investigations about the possible pharmacological role of this compound in brain disturbances, since the rate of transmethylation reactions can be elevated via increased substrate availability in various neurological disorders.

Phospholipid methylation has acquired great interest in recent years (1, 5–9). Conditions, such as aging, decrease the SAMe content in brain (10) and the activity of the methylation pathway in microsomes and synaptic membranes (11, 12).

These data indicate that many factors can affect the extent and function of this pathway in brain. Among these, experimental ischemia is known to produce release of free fatty acids (FFA) at the expenses of various phospholipids (13). The disrupted architecture of brain membranes, due to decrease in concentration and/or turnover of their phospholipids, is also important, since normal brain function depends on intact and functional membranes.

It is not known whether SAMe levels in ischemic brain are decreased, although presumably they should be lower due to decreased energy charge (14). If anyway choline glycerophospholipids (CGP) are degraded during ischemia, and their concentrations decreased, then the administration of SAMe may favor PE methylation to PC and membrane recovery, by causing a positive effect on transmethylation processes via increased substrate availability. In the work described here we have attempted to use SAMe to reverse the lipid changes accompanying an ischemic episode.

EXPERIMENTAL PROCEDURE

Gerbils (*Meriones unguiculatus*), of 50–60 g body wt., were obtained from Donald Robinson (Tumble Brook, Massachusetts) and caged individually. This experimental model

has been reported in details elsewhere (15, 16). Briefly, the gerbil common carotid arteries were exposed after a subcutaneous injection of 1% novocaine (50 μl), and occlusion of both arteries was performed after complete recovery from anesthesia. Sham surgery was carried out on all controls. Anesthesia was avoided for injection of lipid precursors (15, 16). Neither mortality nor tonic-clonic seizures were observed throughout the experiments. Assays have been made of the chief high-energy phosphates and related compounds in the brain of the gerbils at different time intervals from ligation to monitor each time the changes due to ischemia (15). A noticeable reduction of glucose and glycogen levels concurrently with an elevation of those of lactate occurred immediately. Similarly, a noticeable decrease of ATP and phosphocreatine content was observed with a concomitant increase of AMP. These data support the concept that carotid artery occlusion on both sides was effective and that the energy status of the brain was certainly compromised (15).

Sampling of tissue, administration of isotope (intraventricular injection of 0.5 μCi of [1-^{14}C]arachidonic acid, 7–8 nmol, specific activity 60, from The Radiochemical Centre, Amersham, England), extraction of lipid, analysis of FFA and neutral lipid, and estimations of polar lipids were carried out essentially as described in details elsewhere (15, 16). To minimize thawing, all manipulations of the frozen brain (liquid nitrogen) were done with the sample kept in contact with liquid nitrogen, following previously reported suggestions (17). All checks and control experiments were described in details elsewhere (15, 16).

Normally, only 10 min of complete carotid occlusion have been used. Briefly, the gerbils were first injected intraventricularly with labeled arachidonate (15, 16) 2 hr prior to ligation, and then killed 10 min after ligation by dropping the head into liquid nitrogen. Sham-operated gerbils, with and without the injected SAMe (see later), were treated similarly at the same time intervals.

Three types of SAMe treatment were adopted. In the first (experiments *a*), 2 hr after isotope administration the gerbils were given 1.6 mg SAMe (free base)/Kg body wt., intraventricularly (4 μl). SAMe was freshly dissolved each time. Carotid ligation was performed after 10 min from SAMe injection. Immediately after ligation, a successive administration of SAMe in similar amounts was performed intraventricularly, and after 10 min gerbils were killed by dropping the head into liquid nitrogen. Controls were similarly treated with 0.9% NaCl in place of SAMe. In the second type of experiments (experiments *b*), 1 hr after isotope administration the gerbils were given 200 mg freshly dissolved SAMe (free base)/Kg body wt., intraperitoneally (3) in 0.9% NaCl. Carotid ligation was performed 1 hr after SAMe dosing, i.e., 2 hr after isotope administration. 10 min after ligation gerbils were sacrificed as above. Controls were run with NaCl in place of SAMe. Finally, in the third experiments (experiments *c*) SAMe was injected for two weeks by the subcutaneous route(40 mg/Kg body wt., daily). Immediately after the last dose, labeled arachidonate was given as usual, and carotid ligation performed as described 2 hr after isotope administration. Gerbils were then killed 10 min after ligation.

Separation of polar lipids was performed as described elsewhere (15, 16). For neutral lipids, silica gel G plates (0.3 mm of thickness) were developed first with a benzene-ethyl ether-ethyl alcohol-acetic acid (50:40:2:0.2, v/v) solvent system, and then with petroleum ether (70°-80°)-ethyl ether-acetic acid (70:30:1, v/v) as the second. Mass and radioactivity analyses were performed as reported previously (15, 16), as well as GLC assays on the lipid samples. Protein was determined according to Lowry et al. (18). Phospholipid P was estimated following the procedure of Ernster et al. (19) on extracts or TLC spots.

Unless otherwise stated, two similarly-treated gerbils (either controls or not) were constantly used for each experiment and for each time. The determination for each point was carried out in duplicate and the results mediated. The single values of phospholipid P or radioactivity content after TLC and those of neutral lipids were not corrected for recovery,

which however was estimated and reported in each Table, when necessary. An analysis of the variance was carried out, and the values were compared with sham data or others at similar postoperational times. The Student *t*-test was used for statistical comparison.

[1-^{14}C]Arachidonic acid was purchased from the Radiochemical Centre (Amersham, England), [*methyl*-^{14}C]SAMe (53 mCi/mmole) was obtained from similar source. The stable and soluble SAMe disulfate di-*p*-toluenesulfonate salt was obtained from BioResearch Labs. (Milan, Italy). This material was analyzed by high pressure liquid chromatography, in order to exclude possible contaminations, following a previously described procedure (20). The compound was found to be about 98% pure, and no S-adenosyl-homocysteine was found, as checked with the retention times of the standard reference compound (Sigma Chemical Co., St. Louis, Missouri).

RESULTS AND DISCUSSION

Generals. In this contribution only modifications occurring after short times following ligation have been examined, since the main interest of the present work was to investigate on the rapid biochemical changes following ischemia. As mentioned under Experimental Procedure, all gerbils displayed the characteristic signs of energy status due to ischemia. All animals, of any experimental group, survived up to 10 min from bilateral ligation and frequently showed the initial neurological signs of ischemia reported elsewhere (21). Sham-surgery had little effect on the concentration of high energy phosphates and glycolytic compounds in brain, and, therefore, the values in sham animals were similar to those of normal non-operated gerbils. No EGG recording was made of these animals during ligation.

Effect of Intraventricularly-Injected SAMe. This section deals with the experiments (*a*) reported under Experimental. Ischemia produced a statistically significant increase of the FFA pool in the gerbil brain, both of the total and of the main individual FFA (Table I, column B), confirming previous results (15, 16, 22). The concentration of 18:0, 20:4 N-6, and 22:6 N-3 is about 3-fold higher than in the brain of control, sham-operated gerbils (Table I, column A). Much care was taken to avoid any significant blood contamination of the brain FFA pool, and this point has been documented elsewhere (16).

The intraventricular (i.v.) injection of SAMe had no effect on the total and individual FFA concentration of ischemic brain (Table I, column D). This result indicates that if methylation occurred, it did not influence the mechanisms of FFA release in ischemia.

Table II reports data on the radioactivity content of free 20:4 in the gerbil brain during ischemia (column B), together with correspondent values obtained following SAMe injection in both sham-operated (column C) and ligated gerbils (column D). The lack of effect of SAMe upon this

TABLE I

EFFECT OF S-ADENOSYL-L-METHIONINE (SAME) ON FREE FATTY ACID, DIGLYCERIDE, AND POLAR LIPID CONTENT OF ISCHEMIC BRAIN IN THE GERBIL[a]

Lipid	Experimental Groups[b]			
	A	B	C	D
Free fatty acid				
total	327 ± 28.2	951 ± 20.3[d]	367 ± 26.2	923 ± 18.6
18:0	103 ± 23.7	280 ± 11.6[d]	121 ± 7.8	262 ± 10.6
20:4	84 ± 7.0	249 ± 9.8[d]	83 ± 5.9	245 ± 9.0
22:6	12 ± 1.4	31 ± 2.1[d]	14 ± 1.8	27 ± 1.5
Diglyceride				
total	276 ± 11.3	362 ± 30.1[e]	291 ± 9.9	379 ± 27.2
20:4	121 ± 4.4	163 ± 7.2[e]	143 ± 9.2	193 ± 8.0[h]
Phospholipid[c]				
total	47.4 ± 1.02	43.0 ± 0.98[f]	47.0 ± 1.51	45.6 ± 0.75
PI	2.10 ± 0.20	1.57 ± 0.04[f]	2.11 ± 0.16	1.54 ± 0.08
PE	9.29 ± 0.51	9.34 ± 0.62	8.87 ± 0.48	8.78 ± 0.42
PC	17.8 ± 0.72	13.9 ± 0.51[g]	18.4 ± 0.64	17.1 ± 0.52[i]
EP	8.49 ± 0.41	8.44 ± 0.60	8.26 ± 0.45	8.17 ± 0.42

[a] Determinations performed by GLC (15). Phospholipid P and related classes analyzed by TLC (15, 16). The data of FFA and diglyceride are expressed as nmol/g wet wt ± SEM (six experiments) and those of phospholipid as μmol/g wet wt ± SEM (six experiments). See text for other details and for abbreviations.

[b] A = control, sham-operated gerbils; B = ligated, ischemic gerbils; C = control, sham-operated gerbils, receiving SAMe intraventricularly; D = ligated, ischemic gerbils, receiving SAMe intraventricularly. See text for the adopted doses of SAMe.

[c] This value represents total separated polar lipids after TLC (15, 16). The total lipid extract before TLC contained 60.1 μmol/g wet wt. Recovery values were around 80%.

[d] $P < 0.001$ (versus controls, group A); [e] $0.01 > P > 0.05$ (versus controls, group A); [f] $0.05 > P > 0.025$ (versus controls, group A); [g] $0.01 > P > 0.005$ (versus controls, group A); [h] $0.05 > P > 0.025$ (versus B group); [i] $0.01 > P > 0.05$ (versus B group). PI = phosphatidylinositol; PE = phosphatidylethanolamine; PC = phosphatidylcholine; EP = ethanolamine plasmalogen.

parameter is similar to what was reported for unlabeled free 20:4 (Table I).

Ischemia also induced a 31% increase of diglyceride concentration after 10 min of ligation (Table I). The content of 20:4 in the diglyceride fraction also increased by 35% during the same interval, confirming recent data (15, 16). Previous work (15, 16) had indicated that the increase in the concentration of diglyceride and of its 20:4 content could be due both to phospholipase C activation and to stimulation of the reverse reaction catalyzed by the choline phosphotransferase (EC 2.7.8.2). The increase of the diglyceride content is well comparable with the data presented in Table

TABLE II

EFFECT OF S-ADENOSYL-L-METHIONINE (SAMe) ON FREE ARACHIDONATE, DIGLYCERIDE (20:4), AND POLAR LIPID (20:4) RADIOACTIVITY IN ISCHEMIC BRAIN OF GERBIL[a]

Lipid	Experimental Groups[b]			
	A	B	C	D
Arachidonate	2.59 ± 0.08	7.80 ± 0.52[c]	3.06 ± 0.12	7.33 ± 0.66
Diglyceride	3.38 ± 0.32	5.01 ± 0.47[d]	3.80 ± 0.16	5.86 ± 0.60[g]
Total polar lipid	101.5 ± 3.90	73.5 ± 4.80[e]	100.3 ± 5.10	90.2 ± 5.21[g]
PI	21.4 ± 1.00	14.6 ± 1.23[e]	20.9 ± 1.42	15.5 ± 0.60
PE	14.4 ± 0.52	10.6 ± 0.50[e]	12.1 ± 0.71	12.2 ± 0.48
PC	45.8 ± 1.16	32.9 ± 1.13[f]	49.1 ± 1.60	43.5 ± 1.52[h]
EP	5.42 ± 0.32	4.71 ± 0.36	4.48 ± 0.40	4.62 ± 0.33
CP	3.65 ± 0.18	2.93 ± 0.23[d]	3.92 ± 0.26	3.71 ± 0.33[i]

[a] Data refer to mean values (six experiments expressed as nCi/g wet wt ± SEM. Preliminary experiments have indicated that lipid radioactivity was due only to 20:4, as checked by the GLC of the separated fatty acids of the FFA fraction or by silver-staining TLC and successive GLC of the separated fatty acids (15, 16). Labeled species were only those containing 20:4. Recovery values for 20:4 and diglyceride over the radioactivity of the total lipid extract (178.1 ± 9.40 nCi/g wet wt for six experiments) was on 86%, while that for polar lipids over the lipid extract (15, 16) after Bio-Sil A chromatography (126 nCi/g wet wt for six experiments) was 80%. See the text for other details and for abbreviations.

[b] See Table I for the explanation of the groups.

[c] $P < 0.001$ (versus controls, group A); [d] $0.05 > 0.025$ (versus controls, group A); [e] $0.01 > P > 0.05$ (versus controls, group A); [f] $0.01 > P > 0.005$ (versus controls, group A); [g] not significant (versus B group); [h] $0.01 > P > 0.05$ (versus B group); [i] $0.05 > P > 0.025$ (versus B group).

II, which also show the increase of the diglyceride radioactivity during transient ischemia. Ischemia produced in fact a 48% increase of the 20:4 radioactivity (Table II, column B), which correlates well with the 35% increase of the unlabeled 20:4 content of diglyceride (Table I, column B). It must be mentioned here that the lipid radioactivity of the diglyceride fraction was checked to be linked entirely to arachidonate, as established with previous experiments (15, 16).

The i.v. administration of SAMe during ischemia had some effect on the diglyceride concentration. A 18% increase of the 20:4 level of diglyceride was observed (compare columns B and D of Table I). A smaller effect was also detected on the total pool size of diglyceride. These results are comparable with those presented in Table II, where an increase of 17% of the 20:4 radioactivity of diglyceride was noticed (compare column B with D), although not significant. These data indicate that SAMe treat-

ment may influence the synthesis of lipid highly susceptible to further hydrolysis to diglyceride.

Table I also reports the data about polar lipid concentration of gerbil brain during transient ischemia. Only phosphatidylinositol (PI), PE, PC and ethanolamine plasmalogen (EP) were considered, since these lipids are those most effectively influenced by ischemia (15, 16, 22). Ischemia produced decreases of the PI (26%) and PC (22%) concentration (Table I, column B). These findings correlate with those reported for the 20:4 radioactivity content in the polar lipids (Table II, column B), where the labeling of PI, PC, PE, and choline plasmalogen (CP) decreased by 32%, 28%, 26%, and 20%, respectively. A smaller decrease was noticed for EP. The increase of the lysophospholipid concentration reported to occur during transient ischemia (15, 16), as well as the increase of diglyceride (15, 16 and this work) point to the involvement during ischemia of phospholipases A and C for the degradation of the polar lipid classes. In addition, PC may be degraded through the stimulation of the reverse reaction catalyzed by the choline phosphotransferase producing diglyceride and CDP-choline (23).

The effect of the i.v. injection of SAMe on polar lipids is reported in Tables I and II. The treatment prior to ligation raised nearly to normal values those of PC (compare columns B and D of Table I). The same result was obtained for the radioactivity content of PC and CP after similar treatment (compare columns B and D of Table II). The radioactivity levels of PE, which were noticeably decreased by 26% by ischemia were still lower than those of controls after SAMe treatment, although increased compared to the ischemic group (compare columns B and D of Table II). SAMe was unable to reverse the downward trend of PI. Also the total phospholipid P concentration and total lipid radioactivity partially reverted to control values with the same treatment.

Concluding, no significant differences were detected in the degree of CGP labeling and content between control and ischemic brain when the gerbils had the i.v. injection of SAMe. The labeling of PE was also increased following SAMe treatment, still being below the control values. Probably, the increase of the methylation activity due to SAMe administration might lower the radioactivity content of PE at the expenses of the methylated compounds (chiefly PC), though a certain degree of resynthesis of PE still takes place at the same intervals. No influence of SAMe is exerted upon PI metabolism and no effect upon FFA release.

Effect of Intraperitoneally-injected SAMe. Tables III and IV show on the whole that the intraperitoneal (i.p.) injection of SAMe did not influence the total and individual FFA concentration or radioactivity of the

TABLE III
EFFECT OF S-ADENOSYL-L-METHIONINE (SAMe) ON FREE FATTY ACID, DIGLYCERIDE, AND POLAR LIPID CONTENT OF ISCHEMIC BRAIN IN THE GERBIL[a]

Lipid	Experimental Groups[b]			
	A	B	C	D
Free fatty acid				
total	342 ± 26.0	911 ± 18.0[d]	381 ± 30.6	890 ± 19.1
18:0	93 ± 9.0	256 ± 11.1[d]	106 ± 6.0	266 ± 11.3
20:4	81 ± 3.3	236 ± 8.0[d]	74 ± 6.2	215 ± 15.5
22:6	13 ± 1.1	27 ± 1.4[d]	14 ± 1.1	30 ± 1.3
Diglyceride				
total	260 ± 9.6	348 ± 28.6[e]	272 ± 48.1	340 ± 27.1
20:4	111 ± 4.4	156 ± 9.6[e]	126 ± 9.0	169 ± 10.2
Phospholipid[c]				
total	48.3 ± 1.19	42.1 ± 1.01[f]	47.2 ± 1.33	43.9 ± 0.69
PI	2.02 ± 0.26	1.52 ± 0.06[f]	2.20 ± 0.20	1.48 ± 0.10
PE	9.36 ± 0.43	8.90 ± 0.52	9.04 ± 0.40	8.71 ± 0.36
PC	18.1 ± 0.63	12.7 ± 0.49[g]	18.5 ± 0.51	15.8 ± 0.46 [h]
EP	8.52 ± 0.44	8.12 ± 0.51	8.38 ± 0.32	8.10 ± 0.40

[a] The data refer to five experiments expressed as reported in Table I. See the text and Table I for details and abbreviations.
[b] A = control, sham-operated gerbils; B = ligated, ischemic gerbils; C = control, sham-operated gerbils, receiving SAMe intraperitoneally; D = ligated, ischemic gerbils, receiving SAMe intraperitoneally. See text for the adopted doses of SAMe.
[c] This value represents total separated polar lipids after TLC (15, 16). The total lipid extract before TLC contained 59.3 μmol/g wet wt. Recovery values were on 81%.
[d] $P < 0.001$ (versus controls, group A); [e] $0.01 > P$ 0.05 (versus controls, group A); [f] $0.05 > P > 0.025$ (versus controls, group A); [g] $0.01 > P > 0.005$ (versus controls, group A); [h] $0.05 > P > 0.025$ (versus B group).

ischemic brain. These results are in line with those reported in the previous section.

The increase of the diglyceride pool and of its 20:4 content and radioactivity, which was produced by ischemia (column B, Tables III and IV), was not corrected by the treatment (columns D of Tables III and IV). These results are slightly different from those following the i.v. injection of the same compound (see previous section), which indicated some effect of SAMe on the diglyceride pool of brain. Probably, the active SAMe concentrations which reach the brain by the two types of administration are different, and this point will be further exploited.

Table II reports the data about polar lipid concentration of gerbil brain after the i.p. administration of SAMe. The treatment prior to ligation increased noticeably the PC concentration (compare columns B and D of

TABLE IV

EFFECT OF S-ADENOSYL-L-METHIONINE (SAME) ON FREE ARACHIDONATE, DIGLYCERIDE (20:4), AND POLAR LIPID (20:4) RADIOACTIVITY IN ISCHEMIC BRAIN OF GERBIL[a]

Lipid	Experimental Groups[b]			
	A	B	C	D
Arachidonate	2.70 ± 0.12	7.56 ± 0.44[c]	3.11 ± 0.15	7.20 ± 0.61
Diglyceride	3.20 ± 0.27	5.51 ± 0.42[c]	3.31 ± 0.15	5.60 ± 0.49
Total polar lipid	102.7 ± 4.21	77.7 ± 4.62[d]	107.2 ± 6.01	85.9 ± 3.91[g]
PI	20.6 ± 0.88	14.1 ± 1.11[d]	18.9 ± 1.40	14.8 ± 0.70
PE	14.3 ± 0.45	9.9 ± 0.46[e]	12.8 ± 0.62	12.6 ± 0.41
PC	46.2 ± 1.12	33.0 ± 1.15[e]	48.9 ± 1.48	41.7 ± 1.60[h]
EP	5.02 ± 0.24	4.60 ± 0.32	4.52 ± 0.42	4.71 ± 0.28
CP	3.41 ± 0.16	2.81 ± 0.21[f]	3.80 ± 0.27	3.44 ± 0.28[h]

[a] The data refer to five experiments expressed as reported in Table II. See the text and Table II for details and abbreviations. Recovery values for 20:4 and diglyceride over the radioactivity of the total lipid extract (173.4 ± 7.11 nCi/g wet wt for five experiments) was on 84%, while that for polar lipids over the lipid extract (15, 16) after Bio-Sil A chromatography (132 nCi/g wet wt for five experiments) was 78%.

[b] See Table III for the explanation of the groups.

[c] $P < 0.001$ (versus controls, group A); [d] $0.01 > P > 0.05$ (versus controls, group A); [e] $0.01 > P > 0.005$ (versus controls, group A); [f] $0.05 > P > 0.025$ (versus controls, group A); [g] not significant (versus B group); [h] $0.05 < P > 0.025$ (versus B group).

Table III), bringing almost back to control values the PC concentration. The same result was obtained for PC and CP radioactivity content (compare columns B and D of Table IV). The levels of labeled PE, which were decreased during ischemia, were also increased noticeably although not completely.

On the whole, the i.p. SAMe treatment exerts a noticeable effect on the polar lipid concentration and radioactivity following ischemia, channelling presumably the PE into the methylation pathway with further PC synthesis. This applies also to choline plasmalogen (CP), which is known to be formed by methylation of the ethanolamine analogue in brain (8). Finally, also the total phospholipid P content and total lipid radioactivity were partially corrected by this treatment.

Effect of Subcutaneously-Injected SAMe. On performing experiments (*c*) (see Experimental), it has never been possible to detect any effect due to SAMe administration. The noticeable release of FFA due to ischemia was not corrected nor was the increased production of labeled diglyceride. In addition, the treatment was unable to reverse the downward trends of

TABLE V
CONCENTRATION OF S-ADENOSYL-L-METHIONINE (SAME) IN THE GERBIL BRAIN AFTER ITS ADMINISTRATION

Treatment (source)	SAMe (μg/g wet wet ± SEM)	*P*
Control[a]	12.3 ± 0.3 (8)	
Intraventricular[a]	41.4 ± 2.8 (8)	<0.001
Intraventricular[b]	38.3 ± 2.0 (8)	<0.001
Control[c]	11.9 ± 0.5 (8)	
Intraperitoneal[c]	19.8 ± 1.6 (6)	<0.005
Intraperitoneal[d]	14.0 ± 0.9 (6)	n.s.
Control[e]	11.4 ± 0.4 (5)	
Subcutaneous[e]	12.3 ± 0.5 (5)	

[a] Gerbils were given 1.6 mg SAMe/Kg body wt (4 μl). Carotid ligation was performed after 10 min from SAMe administration. After ligation, a successive similar SAMe administration was performed. Gerbils were killed 10 min after second injection. Controls were treated with similar amounts of 0.9% NaCl.
[b] Same as (a), except that gerbils were killed 15 min after second injection.
[c] Gerbils were given 200 mg SAMe/Kg body wt. Carotid ligation was performed 1 hr after SAMe administration. Gerbils were killed 10 min after carotid ligation. Controls were treated with similar amounts of 0.9% NaCl.
[d] Same as (c), except that gerbils were ligated 2 hr after SAMe administration.
[e] Gerbils were given 40 mg SAMe/Kg body wt, daily, for two weeks. Immediately after the last dose, gerbils were ligated as reported in the text. Gerbils were killed 10 min after ligation. Controls were treated with similar amounts of 0.9% NaCl.
All brain samples were immediately removed after sacrifice, well-washed repeatedly with chilled saline, and blotted carefully in filter paper. SAMe content was estimated essentially following previously reported procedures (2–4).

all polar lipid classes. Due to the lack of response of SAMe, the corresponding data have not been tabulated.

Content of SAMe in Brain After Its Administration. The results reported in this work show that SAMe administration to gerbils prior to ligation is able to counteract some of the biochemical changes due to ischemia, although the increase of the free FFA was not reversed. Interestingly, the i.v. and i.p. administrations were rather effective on the polar lipid changes, but the repeated s.c. injection of the same compound was unable to exert significant effects in this connection.

On the light of previous results (3, 4), a study has been therefore undertaken to demonstrate whether the administration of doses of SAMe which have been adopted throughout our work would elevate the endogenous pool of the compound in brain. Table V demonstrates that the i.v. injection of 1.6 mg/Kg (twice at each 10 min interval) of SAMe increases 3.5-fold the levels of that compound in treated ischemic animals (groups

D of Tables I–IV) compared to ischemic gerbils (groups B of Tables I–IV). The increase occurs almost immediately after the second injection and lasts at least after 5 min. The i.p. injection of 200 mg/Kg of SAMe is also able to increase after 1 hr the basal level of the compound in brain by 70%. This result is in fairly good agreement with a previous work (4). Part of the injected SAMe was still evident in brain after two hr. Table V also demonstrates that repeated s.c. administration of SAMe were unable to elevate significantly its brain levels.

On the light of the results reported in Table V, one can tentatively explain those of Tables I–IV. The possibility to elevate the levels of SAMe in brain through the experiments (*a*) and (*b*) may explain the effects of this compound on brain lipid composition and labeling observed during ischemia (Tables I–IV). On the contrary, SAMe concentration is hardly increased in brain tissue after s.c. administration, with a complete lack of effect on brain lipid metabolism.

CONCLUSION

SAMe is able to counteract almost completely the decreased concentration and radioactivity content of PC which takes place in ischemic conditions. A possible explanation is the rapid increase in methylation of existing ethanolamine phospholipids to both CP and PC, due to elevated substrate availability (SAMe). It is known that ischemia enhances the cellular levels of cytidine-5′-phosphate (CMP) (15). It is worth mentioning in this regard that CMP seems to activate noticeably the transmethylation reactions in brain (24). The increased methylation activity may explain why after SAMe treatment the labeling of PE is still lower than in sham-operated control gerbils (columns C of Tables II and IV) and why the same treatment is able only partially to increase these levels in ischemic treated gerbils (columns D of Tables II and IV). A partial resynthesis of PE in SAMe-treated ischemic gerbils occurs, however, possibly to counteract the PE molecules removed for methylation.

The re-establishment of the PC levels does not allow a concomitant recovery of FFA and diglyceride. These two compounds are in fact still high during ischemia in the treated gerbils, and diglyceride further increases during treatment. Probably, SAMe stimulates the synthesis of the arachidonate-rich species of PC and CP and in turn the further conversion of PC to diglyceride by the reverse reaction catalyzed by the choline phosphotransferase (23). Further work is necessary to determine whether the effects of SAMe are mediated through membrane methyltransferases or other mechanisms.

REFERENCES

1. Mozzi, R., Siepi, D., Aandreoli, V., and Porcellati, G. 1982. Conversion of phosphatidylethanolamine to phosphatidylcholine by the methylation pathway in brain, and relation with the membrane structure. Pages 129–138, *in* Usdin, E., Borchardt, T. T., and Creveling, C. R. (eds.), Biochemistry of S-Adenosylmethionine and Related Compounds, McMillan Press, New York.
2. Baldessarini, R. J., and Kopin, I. J. 1966. S-Adenosylmethionine in brain and other tissues. J. Neurochem. 13:769–777.
3. Schatz, R. A., Stramentinoli, G., and Sellinger, O. Z. 1981. Decreased cerebral catabolism of [^{3}H] Histamine in Vivo after S-Adenosylmethionine administration. J. Pharmacol. Exptl. Therap. 216:118–124.
4. Stramentinoli, G., Catto, E., and Algeri, S. 1977. The increase in S-Adenosylmethionine (SAMe) concentration in rat brain after its systemic administration. Comm. Psychopharmacol. 1:89–97.
5. Mozzi, R., and Porcellati, G. 1979. Conversion of phosphatidylethanolamine to phosphatidylcholine in rat brain by the methylation pathway. FEBS Letters 100:363–366.
6. Blusztajn, J. K., Zeisel, S. H., and Wurtman, R. J. 1979. Synthesis of lecithin (phosphatidylcholine) from phosphatidylethanolamine in bovine brain. Brain Res. 179:319–327.
7. Crews, F. T., Hirata, F., and Axelrod, J. 1980. Identification and properties of methyltransferases that synthesize phosphatidylcholine in rat brain synaptosomes. J. Neurochem. 35:1491–1498.
8. Mozzi, R., Siepi, D., Andreoli, V., and Porcellati, G. 1981. The synthesis of choline plasmalogen by the methylation pathway in rat brain. FEBS Letters 131:115–118.
9. Mozzi, R., Goracci, G., Siepi, D., Francescangeli, E., Andreoli, V., Horrocks, L. A., and Porcellati, G. 1982. Phospholipid synthesis by interconversion reactions in brain tissue. Pages 1–12, *in* Horrocks, L. A., Ansell, G. B., and Porcellati, G. (eds.), Phospholipids in the Nervous System. Vol. I, Raven Press, New York.
10. Elioranta, T. O. 1977. Tissue distribution of S-Adenosyl-methionine and S-Adenosyl-homocysteine in the rat. Effect of age, sex and methionine administration on the metabolism of S-Adenosyl-methionine, S-Adenosyl-homocysteine and polyamines. Biochem. J. 166:521–529.
11. Mozzi, R., Andreoli, V., and Porcellati, G. 1980. Involvement of S-adenosylmethionine in brain phospholipid metabolism. Pages 41–54, *in* Cavallini, D., Gaull, G. E., and Zappia, V. (eds.), Natural Sulfur Compounds: Novel Biochemical and Structural Aspects, Plenum Press, New York.
12. Calderini, G., Aldinio, C., Crews, F. T., Gaiti, A., Scapagnini, U., Algeri, S., Ponzio, F., and Toffano, G. 1981. Aging and information processing. Pages 54–56, *in* Corsini, G. U., and Gessa, G. L. (eds.), Apomorphine and Other Dopaminomimetics. Vol. 2, Raven Press, New York.
13. Trovarelli, G., De Medio, G. E., Dorman, R. V., Piccinin, G. L., Horrocks, L. A., and Porcellati, G. 1981. Effect of cytidine-diphosphate choline (CDP-choline) on ischemia-induced alterations of brain lipid in gerbil. Neurochem. Res. 6:821–833.
14. Siesjö, B. K. 1978. Brain Energy Metabolism, pages 458–566. John Wiley and Sons, New York.
15. De Medio, G. E., Goracci, G., Horrocks, L. A., Lazarewicz, J. W., Mazzari, S., Porcellati, G., Strosznajder, J., and Trovarelli, G. 1980. The effect of transient ischemia on fatty acid and lipid metabolism in the gerbil brain. Ital. J. Biochem. 29:412–432.

16. PORCELLATI, G., DE MEDIO, G. E., FINI, C., FLORIDI, A., GORACCI, G., HORROCKS, L. A., LAZAREWICZ, J. W., PALMERINI, C. A., STROSZNAJDER, J., and TROVARELLI, G. 1978. Phospholipid and its metabolism in ischemia. Pages 285–302, *in* NEUHOFF, V. (ed.), Proceedings of the European Society for Neurochemistry, Vol. I, Verlag Chemie, Weinheim.
17. BAZAN, N. G., JR., BAZAN, H. E. P., KENNEDY, W. G., and JOEL, C. D. 1971. Regional distribution and rate of production of free fatty acids in rat brain. J. Neurochem. 18:1387–1393.
18. LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., and RANDALL, R. J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.
19. ERNSTER, L., ZETTERSTRÖM, R., and LINDBERG, O. 1950. Phosphorus determination by the phosphomolybdate reagent. Acta Chem. Scand. 4:942–950.
20. FLORIDI, A., FINI, C., PALMERINI, C. A., MOZZI, R., and PORCELLATI, G. 1979. High performance liquid chromatographic analysis of S-Adenosylmethionine and S-Adenosylhomocysteine in rat liver. J. Liquid Chromat. 2:1003–1015.
21. KOBAYASHI, M., LUST, W. D., and PASSONNEAU, J. V. 1977. Concentration of energy metabolites and cyclic nucleotides during and after bilateral ischemia in the gerbil cerebral cortex. J. Neurochem. 29:53–59.
22. MARION, J., and WOLFE, L. S. 1979. Origin of the arachidonic acid released post-mortem in rat forebrain. Biochim. Biophys. Acta 574:25–32.
23. GORACCI, G., FRANCESCANGELI, E., HORROCKS, L. A., and PORCELLATI, G. 1981. The reverse reaction of choline phosphotransferase in rat brain microsomes: a new pathway for degradation of phosphatidylcholine. Biochim. Biophys. Acta 664:373–379.
24. MOZZI, R., and PORCELLATI, G. 1982. Unpublished observations.