Biochemical Pharmacology, 1966, Vol. 15, pp. 741–748. Pergamon Press Ltd., Printed in Great Britain.

# ALTERATIONS IN TISSUE LEVELS OF S-ADENOSYLMETHIONINE*

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(*Received* 28 *September* 1965; *accepted* 12 *November* 1965)

**Abstract**—Tissue levels of S-adenosylmethionine (SAMe) were measured by a sensitive and specific isotope derivative, isotope dilution technique. The SAMe concentration in tissues can be altered by decreased production or increased utilization. Methionine administration increases SAMe concentration, while active methyl acceptors, such as pyrogallol, lower tissue levels. Tropolone, purpurogallin, pargyline, and imipramine also appear to lower tissue levels of this active methyl donor. Several environmental and pharmacological variables do not alter SAMe concentrations. Alteration of the activity of methionine-activating enzyme does not appear to be responsible for lowering SAMe levels with pyrogallol, tropolone, or pargyline. It is suggested that increased SAMe utilization is the important factor in the lowering of tissue SAMe by pyrogallol, purpurogallin, and pargyline.

TRANSMETHYLATION is an important means of altering the biological activity of a great many compounds. Methionine, in its active form, S-adenosylmethionine (SAMe), is a major donor of methyl groups in mammalian tissues. SAMe is formed from methionine and ATP in the presence of a specific activating enzyme.[1] It is possible to measure the small amounts of SAMe present in tissues.[2] The effects of several metabolic and pharmacologic variables on tissue levels of SAMe have been studied.

## MATERIALS AND METHODS

*Animals.* Normal adult Sprague-Dawley or Osborne-Mendel albino rats were maintained on Purina laboratory chow *ad libitum*, but were fasted overnight before assay of tissue SAMe levels.

*Assay methods.* S-Adenosylmethionine was measured by a specific and sensitive isotope derivative, isotope dilution technique previously reported[2,3] SAMe was assayed by reducing the specific radioactivity (curies per mole) of S-adenosylmethionine-methyl-$^{14}$C (New England Nuclear Corp., 40 c/mole) with the unlabeled SAMe present in tissue homogenates. The latter were prepared by homogenizing weighed aliquots of fresh, wet, iced tissues in 5–10 volumes of 10% trichloroacetic acid in dilute HCl and centrifuging at 4° to obtain a clear protein-free acid supernatant. SAMe-$^{14}$C was added. The TCA was removed by ether-washing, and the acidic aqueous material was buffered at pH 6·8 with a phosphate-bicarbonate buffer. Next,

* The following abbreviations are used: SAMe, S-adenosylmethionine; NAS, N-acetylserotonin, MAO, monoamine oxidase; COMT, catechol-O-methyl transferase; MAE, methionine-activating enzyme.

$H^3$-N-acetylserotonin[4] (35 c/mole) was added with an aliquot of a preparation[5] of bovine pineal hydroxyindole-O-methyl transferase, for which SAMe is the only known methyl donor present in tissues.[2,5] The product of incubation for 1 hr at 37°, melatonin-acetyl-$^3$H-methoxy-$^{14}$C, was extracted into chloroform after strongly alkalinizing the reaction mixture with 1 N NaOH. The radioactive product was demonstrated chromatographically in solvent systems to occur in a single "peak" of radioactivity at the same $R_f$ as authentic melatonin; eluates of such peaks have the same ratio of $H^3$:$^{14}$C as the chloroform extracts used for chromatography.[2, 3, 5] The ratio of $^3$H:$^{14}$C was measured by liquid scintillation spectrometry in a Packard scintillation counter.[2] Internal standards were not needed, since counting efficiency for both $^3$H and $^{14}$C was constant.

It was found that the radioactivity ratio $^3$H:$^{14}$H bears a demonstrated linear relation to the amount of unlabeled tissue SAMe present.[2, 3] This relationship affords a method of quantitative assay of tissue SAMe, which is presented as micrograms SAMe per gram fresh wet tissue; 1 $\mu$g SAMe = 2·5 m$\mu$moles, and the method can detect less than 1 $\mu$g[2]. Pure crystalline SAMe (California Biochemical Corp.) added to tissue homogenates can be recovered quantitatively. Individual determinations vary by less than 4%.

Levels of SAMe in tissues left intact on ice for as long as 4 hr were unaltered. At 24 hr, significant losses were detected. To avoid alterations in tissue assay values, SAMe-$^{14}$C was added to the homogenates immediately in order to fix the specific radioactivity of the SAMe mixture.

Methionine-activating enzyme was assayed by the synthesis *in vitro* of SAMe-$^{14}$C from L-methionine-$^{14}$C (New England Nuclear Corp., 12·5 c/mole) and ATP, and its isolation on chromatographic columns of Dowex-$NH^+$.[4] The $^{14}$C eluted from the columns by ammonia bears a linear relation to incubation time and to the amount of tissue enzyme preparation used. This sensitive method is described in detail elsewhere.[6]

*Metabolic and pharmacologic experiments.* Rats were examined under a variety of conditions, including exposure to a 4° cold room overnight; injection of 0·9% saline (1 ml, i.p.); and treatment with thyroxin for 4 days (0·1 mg/day, i.m.). Adrenal SAMe was measured 3 hr after a dose (2·5 mg/kg, s.c.) of crystalline insulin. Other rats were given injections of *d,l*-methionine (100 mg/kg, i.p.).

Several compounds expected to alter the utilization of SAMe were tested. They were nicotinamide, pyrogallol, and purpurogallin (K & K Laboratories, Plainview, N.Y., 100 mg/kg, i.p.), and tropolone (Aldrich Chemical Co., Milwaukee, Wis., 25 mg/kg, i.p.).

Several monoamine oxidase inhibitors were surveyed for possible effects on tissue SAMe (iproniazid, 50 mg/kg, i.p.; pheniprazine, 10 mg/kg, i.p.; tranylcypromine, 10 mg/kg, i.p.; and pargyline, 50 mg/kg, i.p.). The degree of MAO inhibition obtained was determined by an assay of MAO activity.[7]

The following neuropharmacologic agents were also studied: chlorpromazine (20 mg/kg, i.p.), imipramine (15 mg/kg, i.p.), reserpine (2·5 mg/kg, i.p.), pentobarbital (2·5 mg/kg, i.p.), and *d*-amphetamine (25 mg/kg, i.p.). Drug combinations (reserpine with pheniprazine, reserpine with methionine, pargyline with methionine) were also examined.

The drugs were given in 1 ml of 0·9% saline at 24 hr and 1–2 hr before sacrifice, unless otherwise specified. Except for an initial study of normal and methionine-

treated rats, only liver and brain were assayed for SAMe. The animals were healthy adult rats weighing 200–250 g, except as noted.

## RESULTS AND DISCUSSION

*Methionine administration*

Thirty minutes after injection of methionine, tissue levels of SAMe were elevated (Table 1). These findings indicate that synthesis of SAMe may be made to exceed its utilization under conditions of precursor loading. Rat liver and brain can convert methionine to SAMe very rapidly,[8] and the necessary activating enzyme is widely distributed among tissues.[6]

TABLE 1. TISSUE CONTENT OF SAMe AFTER TREATMENT WITH METHIONINE

$\mu$g/g, mean ± S.E.M.

| Tissue | Control | N | Methionine-treated | N | *P* |
|---|---|---|---|---|---|
| Adrenal* | 48·0 ± 10·0 | 8 | 61·0 ± 2·0 | 2 | |
| Liver† | 26·0 ± 1·0 | 17 | 115·0 ± 15·0 | 10 | <0·001 |
| Heart* | 25·7 ± 6·2 | 8 | 42·8 ± 0·6 | 2 | |
| Spleen* | 24·0 ± 4·2 | 8 | 33·7 ± 0·1 | 2 | |
| Kidney* | 20·4 ± 5·4 | 8 | 33·8 ± 0·2 | 2 | |
| Lung* | 11·1 ± 2·2 | 8 | 24·5 ± 1·3 | 2 | |
| Brain† | 11·5 ± 0·5 | 12 | 15·0 ± 0·6 | 6 | <0·001 |

* Thirty min after 100 mg *l*-methionine/kg, i.p., in 250-g Sprague-Dawley male rats.

† Thirty min after 100 mg *dl*-methionine/kg, i.p., in 200-g Sprague-Dawley female rats (with matched and unmatched controls accumulated, since they did not differ significantly).

N = number of animals, *P* = statistical probability by Student's *t*-test, throughout tables.

TABLE 2. EFFECT OF DRUGS THAT ALTER SAMe UTILIZATION ON RAT TISSUE LEVELS OF SAMe

$\mu$g/g, mean ± S.E.M.

| Drug | Liver | | | Brain | | |
|---|---|---|---|---|---|---|
| | Control | Treated | *P* | Control | Treated | *P* |
| Nicotinamide | 25·1 ± 3·1 | 27·9 ± 2·8 | <0·6 | | | |
| Pyrogallol | 28·0 ± 1·0 | 8·5 ± 0·5 | <0·001 | 12·0 ± 0·7 | 3·0 ± 0·6 | <0·001 |
| Purpurogallin | 25·5 ± 1·5 | 9·0 ± 2·0 | <0·001 | 12·0 ± 0·5 | 8·5 ± 0·5 | <0·5 |
| Tropolone | 25·1 ± 3·1 | 15·3 ± 1·9 | <0·02 | | | |

N = 6 adult Sprague-Dawley female rats.

*Drugs that alter SAMe utilization*

Nicotinamide is known to accept methyl groups from SAMe in the presence of rat liver extracts,[9] but it had no effect on SAMe levels in liver (Table 2) in the doses used, nor in liver or brain when given for 1 hr as an i.v. infusion of 100 mg/kg.

Pyrogallol is also known to accept methyl groups,[10] and it lowered SAMe strikingly in liver and brain at a dose of 100 mg/kg, i.p. (Table 2). A lower dose (25 mg/kg, i.p.) did not alter liver SAMe levels, however. Pyrogallol is not only a methyl acceptor

but, in doses of 100 mg/kg, it is also an inhibitor of COMT.[11] It prolongs the pharmacologic effects of administered catecholamines.[12] Pyrogallol may reduce the effectiveness of methylation as a means of inactivating catecholamines by accepting methyl groups and lowering SAMe levels, and thus depriving other methyl acceptors of SAMe, as well as inhibiting COMT.

Tropolone also inhibits COMT[13] and if it is not readily methylated, it might be expected to decrease SAMe utilization and possibly to *elevate* SAMe. When tropolone was administered at 100 mg/kg to rats, the animals died within several hours. Doses of 25 mg/kg, however, were not grossly toxic, and produced significant *decreases* of tissue SAMe (Table 2).

Purpurogallin, a compound which is both a tropolone and a triphenol (thus resembling both tropolone and pyrogallol), also lowered tissue SAMe (Table 2).

Pyrogallol, tropolone, and purpurogallin were incubated with the supernatant solution of rat liver homogenate and $^{14}$C-SAMe (Table 3). Only pyrogallol and purpurogallin appear to have accepted $^{14}$C-methyl to any significant extent. The appearance of some organic solvent-extractable $^{14}$C-"methylated products" in the absence of added substrate (Table 2) has been reported previously.[13]

Monophenolic compounds may be methylated by SAMe and liver microsomes after preliminary hydroxylation to catechols,[14] or even directly as phenols.[15] Tropolone was incubated with liver microsomes and $^{14}$C-SAMe by the methods outlined in Table 3. Again, tropolone failed to increase the yield of organic solvent-extractable $^{14}$C-methyl.

TABLE 3. TRANSFER OF $^{14}$C-METHYL FROM SAMe TO METHYL ACCEPTORS

| Methyl acceptors | $C^{14}$* (counts/min) |
|---|---|
| Pyrogallol | 5575 |
| Purpurogallin | 5860 |
| Tropolone | 600 |
| No acceptor | 532 |
| Blank (pyrogallol + tropolone, omit liver extract) | 19 |

* $^{14}$C represents material extracted into chloroform from an aqueous phase made strongly acid after incubating in duplicate at 37° for 90 min, 40 mμc S-adenosylmethionine-methyl-$^{14}$C, 20 μmoles methyl acceptor (in 2·5% sodium carbonate–normal saline), and 2 ml 0·67 M (pH 7·4) phosphate buffer; in the presence of 0·5 ml supernatant solution of a homogenate (in 5 volumes 1·15% KCl) of 2·0 g rat liver prepared at 4° and centrifuged at 48,000 *g* for 30 min. The $^{14}$C thus represents transferred methyl groups attached to an acceptor molecule.

Pyrogallol (100 mg/kg) and tropolone (25 mg/kg) failed to alter liver methionine-activating enzyme activity (Table 4).

In summary, the lowering of tissue SAMe by pyrogallol and purpurogallin may be explained in terms of increased utilization of SAMe by these methyl acceptors. Nicotinamide can accept methyl groups from SAMe but must do so at a relatively

low rate and thus fail to lower SAMe. The lowering of SAMe by tropolone is not readily explained, and it does not seem to be due to increased utilization of SAMe by transfer of methyl groups to tropolone, or to loss of activity of the methionine-activating enzyme. Since the dose of tropolone used was 25% of a lethal dose, certain unknown metabolic abnormalities affecting SAMe or methionine might result.

TABLE 4. LIVER METHIONINE-ACTIVATING ENZYME ACTIVITY AFTER PYROGALLOL, TROPOLONE, AND PARGYLINE TREATMENT*

| Condition | Liver [MAE] | N | *P* |
|---|---|---|---|
| Untreated | 256 ± 16 | 5 | |
| Pargyline | 232 ± 15 | 4 | >0·3 |
| Pyrogallol | 209 ± 12 | 4 | <0·1 |
| Tropolone | 310 ± 16 | 6 | <0·1 |

* Liver MAE activity after doses of pargyline (50 mg/kg, i.p.), pyrogallol (100 mg/kg, i.p.), or tropolone (25 mg/kg, i.p.) given 20, and 2 hr before sacrifice (means ± S.E.M.). N = number of adult female rats assayed in duplicate; P = probability by *t*-test. A unit of activity is a millimicro-mole of SAMe-$^{14}$C formed in 30 min per g fresh wet liver.

*Monoamine oxidase (MAO) inhibitors*

Pollin *et al.*[16] and others[17–19] have found striking behavioral changes in schizophrenic patients given an MAO inhibitor with methionine, which were not induced by either agent alone. Such findings and the fact that several psychomimetic drugs[20, 21] are methylated amines (e.g. mescaline, harmine, psilocybin, trimethoxyamphetamine) have led to the hypothesis that MAO inhibition coupled with methionine loading (and thus, SAMe elevation) may favor the formation of unique methylated amines of possible significance in inducing certain psychotic behavioral states. Decreased amine oxidation could result in increased use of methylation as a means of amine inactivation. Therefore, a series of MAO inhibitors was examined for their possible effects on SAMe levels. The results (Fig. 1) suggest that no striking effects are produced except in the case of pargyline, a potent nonhydrazine MAO inhibitor, which produced significant decreases in brain and liver levels of SAMe.

When methionine (100 mg/kg, i.p.) was given with pargyline, SAMe levels were less elevated than after treatment with the same dose of methionine alone (Fig. 2). These results indicate that methionine excess antagonizes the lowering of SAMe by pargyline. MAE activity is not significantly altered by pargyline (Table 4). Thus it is probable that pargyline increases methyl donor utilization.

A comparison of the effectiveness of pargyline in inhibition of MAO with that of pheniprazine (which did not lower SAMe levels) in the same doses used to study SAMe revealed (Table 5) that pargyline decreased MAO activity to less than 1% of control, but that pheniprazine was 6–7 times less effective. It is suggested that SAMe lowering by increased utilization may be a function of the completeness of inhibition of amine oxidation.

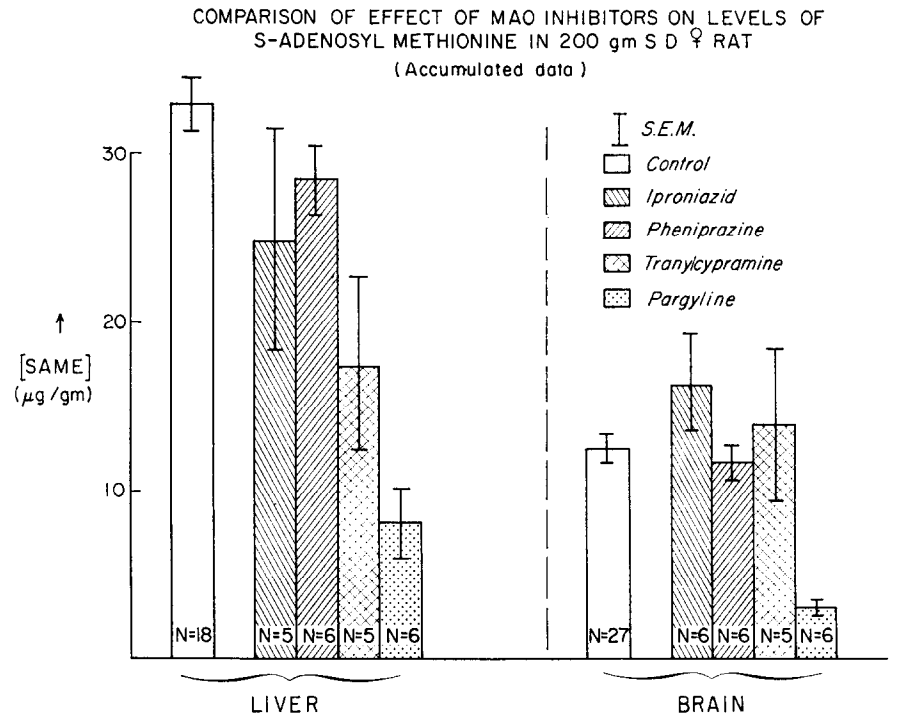


FIG. 1. Survey of monoamine oxidase inhibitors for effects on S-adenosylmethionine in adult Sprague-Dawley female rats.

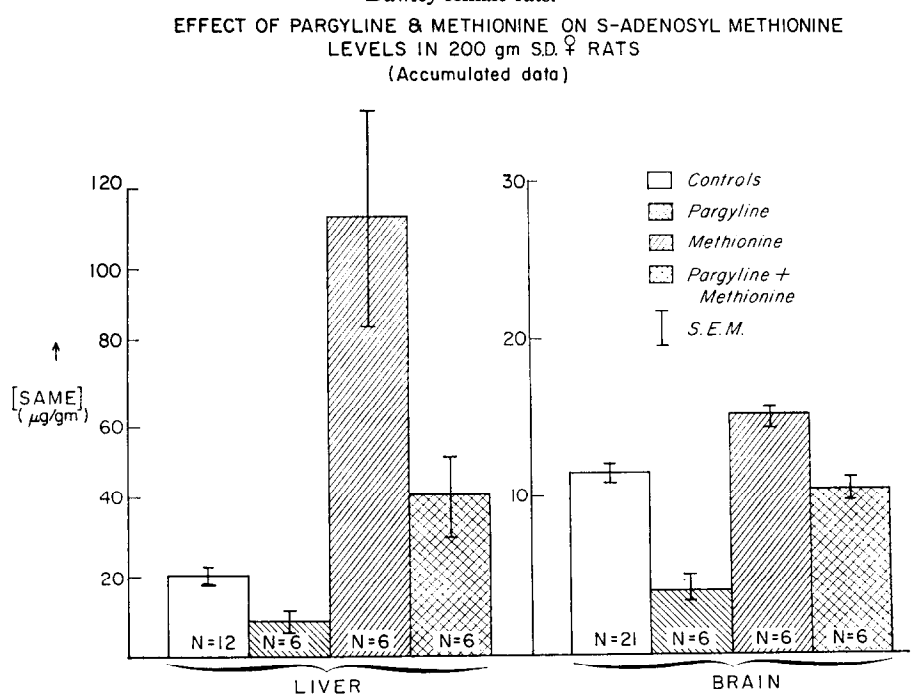


FIG. 2. Effect of methionine in preventing the decrease in SAMe by pargyline.

*Effects of other drugs*

Since one potent antidepressant agent, pargyline, lowered SAMe levels, it was of interest to inquire whether this might be a property of other mood-altering drugs. Imipramine produced variable effects in liver, but produced striking and reproducible lowering of SAMe in rat brain (Table 6). The mechanism of this effect may be related to the observed effects of the drug on brain amines.[22] However, another potent drug *d*-amphetamine, with similar effects on amine metabolism,[23] had no effect on tissue levels of SAMe.

TABLE 5. RELATIVE MAO INHIBITION BY PHENIPRAZINE AND PARGYLINE*

| Tissue | Residual MAO activity (%) | |
|---|---|---|
| | Liver | Brain |
| After pargyline | 0·8% ± 0·2 | 0·6% ± 0·2 |
| After pheniprazine | 7·3% ± 0·7 | 6·2% ± 0·3 |

* Drugs were given 24, and 2 hr before sacrifice, to 5 rats per group in the doses shown in Fig. 1. Results are expressed in terms of per cent of control activity; 100% liver activity is 1016 units ± 63 (mean ± S.E.), and 100% brain activity is 694 units ± 30. A unit of activity is 1 mμmole tryptamine-2-$^{14}$C oxidized to indoleacetic acid-2-$^{14}$C/g wet tissue.

TABLE 6. EFFECT OF IMIPRAMINE ON RAT BRAIN SAMe*

| N | Control (SAMe) | N | Treated (SAMe) | P |
|---|---|---|---|---|
| 10 | 9·0 ± 0·7 | 6 | 5·0 ± 0·3 | <0·001 |

* (SAMe) in μg/g ± S.E.M.
N = number of 125-g Sprague-Dawley female rats.

Two drugs that tend to depress the central nervous system, reserpine and chlorpromazine, produced only small and variable effects on tissue SAMe levels. Furthermore, reserpine did not inhibit the elevation of tissue SAMe by methionine and failed to potentiate the small decrease in SAMe seen after pheniprazine. A third central depressant, pentobarbital, also failed to alter SAMe.

Thyroxin failed to alter SAMe, and may have either no effects at all or may increase both synthesis and utilization.

The effect of ethionine on SAMe levels is not clear. Small doses of ethionine (100 mg/kg, i.p., at 18 and at 2 hr before sacrifice) produced no apparent change in SAMe levels in rat liver or brain. High doses (1000 mg/kg, i.p.) apparently caused striking elevations in SAMe. Such doses interfere with methylation processes and produce high tissue levels of S-adenosyl*ethionine*.[24] The apparent SAMe elevation is no doubt partly artifactual, since S-adenosyl*ethionine* may participate in the enzymatic transalkylation (with partially purified bovine pineal hydroxyindole-O-methyl transferase) used to assay SAMe, with about 20% the efficiency of SAMe[8] itself to give falsely high estimates of SAMe.

Under conditions known to produce hypoglycemia and expected to stimulate epinephrine synthesis[25] (and thus increased utilization of SAMe), there was no significant difference in rat adrenal SAMe or MAE concentration from normal controls. It may be that adrenal SAMe and MAE concentrations (among the highest found in any tissues[2, 6]) are more than adequate to meet increased metabolic demands for methyl transfer.

*Negative studies*

Several other conditions were found to cause no significant differences in tissue SAMe concentration between treated and control rats. There were no significant differences between male and female rats of the same size and strain. Saline injections and cold stress did not alter SAMe levels.

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