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PHYSIOLOGICAL DEPENDENCE OF BRAIN METHIONINE AND *S*-ADENOSYLMETHIONINE CONCENTRATIONS ON SERUM AMINO ACID PATTERN

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Abstract—Animals maintained on rat chow and water *ad libitum* in quarters illuminated for 12 h/day show diurnal rhythms in serum methionine and brain *S*-adenosylmethionine (SAM) concentrations. Brain methionine exhibits no such variation, nor does the ratio of serum methionine to the serum concentrations of six neutral amino acids which are believed to compete with methionine for uptake into brain. Administration of methionine to rats in doses that elevate serum methionine, but keep it within the daily physiological range, significantly increases brain concentrations of both methionine and SAM. The acute feeding of either a protein-free or a 40% casein meal also increases brain methionine and SAM, but does not affect serum methionine; however, both diets also increase the ratio of serum methionine to tyrosine, an amino acid whose postprandial concentration is indicative of the concentrations of the other amino acids that compete with methionine for transport into brain. These findings suggest that brain methionine levels increase physiologically after eating as a result of changes in the serum amino acid pattern. Furthermore, such naturally occurring increases in brain methionine appear to be associated with elevations in brain SAM.

BALDESSARINI (1966) has shown that the concentration of *S*-adenosylmethionine (SAM) in rat brain increases 30 min after the intraperitoneal injection of 100 mg/kg of dl-methionine. Brain SAM concentrations in untreated *ad libitum*-fed rats have also been found to exhibit a daily rhythm (WURTMAN *et al.*, 1970) which roughly parallels the rhythm in plasma methionine concentrations (FERNSTROM *et al.*, 1971). These observations are all compatible with the hypothesis that the rate at which rat brain synthesizes SAM varies physiologically as a function of methionine availability. However, no data are available on actual brain methionine concentrations in either of the above types of experiments.

The problem of the relationship between methionine availability and brain SAM can be reduced to two questions: First, what is the relationship between the concentrations of methionine (and perhaps, other amino acids) in plasma or serum and its concentration in brain, and, secondly, do physiologic changes in brain methionine lead to corresponding changes in brain SAM concentrations? Such a relationship between plasma amino acid pattern and the concentrations in brain of a particular amino acid and its products has already been demonstrated to exist for tryptophan and serotonin (5HT): diet-induced increases in the ratio between plasma tryptophan and other neutral amino acids that compete with tryptophan for brain uptake are quickly followed by increases in the brain concentrations of tryptophan and 5HT (FERNSTROM & WURTMAN, 1972; WURTMAN & FERNSTROM, 1972; FERNSTROM *et al.*, 1973; FERNSTROM *et al.*, 1974). This study examines the physiological control of brain methionine and SAM by serum amino acids.

METHODS

Materials. L-[Methyl-^{14}C]methionine was obtained from Schwarz Bio Research Inc. (New York) (50 mCi/mmol) or from the New England Nuclear Co. (Boston, MA) (41·3 mCi/mmol). [Methyl-^{14}C]-*S*-Adenosyl-L-methionine (53·1 mCi/mmol) was obtained from the New England Nuclear Co. [Acetyl-^{3}H]-*N*-acetylserotonin was prepared from serotonin creatinine sulphate (Regis Chemical Co.,

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Abbreviations used: SAM, *S*-adenosylmethionine; 5HT, 5-hydroxytryptamine.

Chicago, Ill.) and [^{3}H]acetic anhydride (New England Nuclear Co.) and purified by paper chromatography (KOPIN *et al.*, 1961). *S*-adenosyl-L-methionine (grade II, 70 per cent pure) was obtained from the Sigma Chemical Co. (St. Louis, Mo.). Yeast tRNA was purchased from Schwarz Bio Research Inc. Disodium ATP was obtained from Pabst Laboratories (Milwaukee, Wis.). All other chemicals were reagent grade.

Animals. Adult male Sprague–Dawley albino rats (Charles River Breeding Laboratories, Wilmington, Mass.) were housed 12 per cage and maintained on Purina Laboratory Rat Chow and water *ad libitum*, unless otherwise indicated. The animal quarters were illuminated with simulated sunlight (Vita-Lite, 300 μW/cm^2, Duro Test Corp., North Bergen, NJ.) from 9 a.m. to 9 p.m. daily. Except where indicated, each experimental group contained six rats.

L-Methionine, dissolved in 1 ml of 0·9% NaCl, was injected intraperitoneally. All animals were killed by decapitation; blood was collected from the cervical wound, and serum was prepared by centrifugation of the clotted blood. Brain samples were removed within 4 min of death and frozen immediately on dry ice. Some brains were hemisected sagittally prior to freezing. Tissues were stored frozen until they were to be weighed and homogenized. Deproteinized samples for determination of metabolites were prepared by homogenization in 10% trichloroacetic acid dissolved in 0·05 N HCl; samples for assay of methionine adenosyltransferase were homogenized in 0·03 M potassium phosphate buffer, pH 6·9.

Assays. *S*-Adenosylmethionine was measured by the method of BALDESSARINI & KOPIN (1966); all SAM assays were performed in duplicate. Methionine was determined as previously described (ORDONEZ & WURTMAN, 1973). Tyrosine was assayed by the method of WAALKES & UDENFRIEND (1957). Methionine adenosyltransferase (EC 2.1.5.6) was measured by a minor modification of the method of MUDD *et al.* (1965); reduced glutathione was omitted from the incubation mixture, and the final volume was increased to 0·75 ml. Protein was determined by the method of LOWRY *et al.* (1951). Radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer. Aqueous aliquots were counted in 13 ml of naphthalene phosphor, containing 4 g of PPO, 50 mg of POPOP, and 120 g of naphthalene per litre of 1,4 dioxane. Filter paper discs from the methionine assay were counted in 10 ml of toluene phosphor, containing 42 ml of Liquifluor (New England Nuclear Corp.) per litre of toluene. The melatonin residue from the SAM assay was counted in 13 ml of 5 : 2 v/v toluene phosphor–absolute enthanol.

Treatment of data. Data are presented as means plus or minus the standard errors of the mean (S.E.M.). Significance was determined by Student's *t*-test at the 95 per cent confidence level or better.

RESULTS

Rhythm study. Thirty-six rats housed under standard conditions for at least 5 days were killed in groups of six every 4 h, starting at noon. Half of each brain was assayed for methionine and SAM, the other half for methionine adenosyltransferase; serum was assayed for methionine.

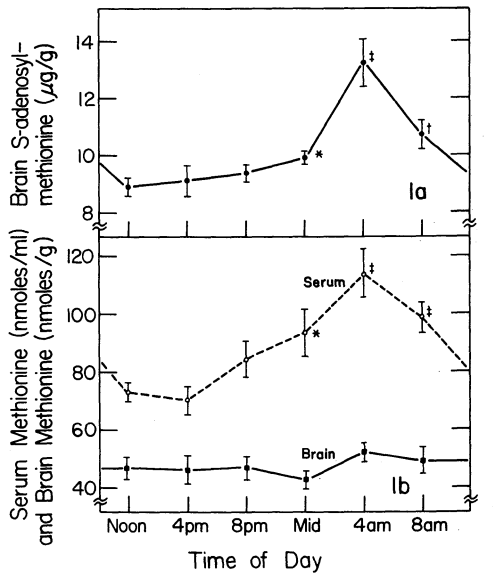

FIG. 1. Brain *S*-adenosylmethionine (1A) and serum and brain methionine (1B) at different times of day. Animals maintained on Purina Laboratory Rat Chow and water *ad libitum* and kept in quarters illuminated from 9 a.m. to 9 p.m. daily were killed in groups of six every 4 h. Statistical significance was determined with respect to nadir values: * $P < 0.05$; † $P < 0.02$; ‡ $P < 0.01$.

The peak serum methionine level, at 4 a.m., was 61 per cent higher than the 4 p.m. nadir (Fig. 1b). These results confirmed previous observations (FERNSTROM *et al.*, 1971), which showed 6 a.m. values 46 per cent higher than those measured at 6 p.m. Brain methionine concentrations failed to exhibit significant variations throughout the day (Fig. 1b). Brain SAM levels, like serum methionine, peaked at 4 a.m., attaining values 48 per cent higher than those observed at noon (Fig. 1a). These findings also confirmed a previous report (WURTMAN *et al.*, 1970) that found mid-darkness brain SAM levels (comparable to those found at 3 a.m. in the present study) to be 50 per cent higher than midday levels (3 p.m.). No significant diurnal fluctuations were detected in brain methionine adenosyltransferase activities.

Injection studies. To determine the extent to which induced increases in serum methionine could modify brain methionine and SAM concentrations, 100 mg/kg of the amino acid was injected intraperitoneally into groups of six rats weighing 150–175 g, and the animals were killed 1, 2, 4 or 8 h later. The injections were timed so that all animals were killed

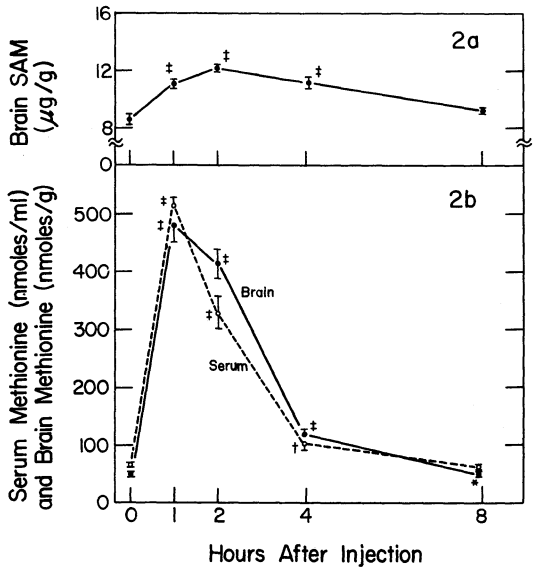

FIG. 2. Brain *S*-adenosylmethionine (2A) and serum and brain methionine (2B) at different times after injection of 100 mg/kg L-methionine. Injections were timed so that all animals would be killed at the same time of day to avoid variations caused by daily rhythms. Significance of differences was determined with respect to control (0 time): * $P < 0{\cdot}02$; † $P < 0{\cdot}01$; ‡ $P < 0{\cdot}001$.

at the same hour of the day, in order to minimize possible effects of daily variations on the fate of the injected material. One hour after methionine was injected, its concentrations in serum and brain attained values about 10 times those found in control animals (Fig. 2b). SAM was maximally elevated (by about 35 per cent) 2 h after methionine injection (Fig. 2b).

The very great increase in serum methionine produced by injecting 100 mg/kg of the amino acid caused its concentration to rise well beyond that normally observed in untreated animals (Fig. 1). To determine whether induced increases in serum methionine that were *within* the physiological range for this amino acid could also increase brain methionine and SAM, groups of six rats weighing 200–300 g were injected with 10, 30 or 100 mg/kg of the amino acid and killed 2 h later (i.e. at the time of peak brain SAM concentrations); control animals received no injection. At the lowest dose, none of the variables measured was significantly elevated above the control value. The serum methionine concentration of rats receiving 30 mg/kg of methionine was significantly elevated (Table 1), but remained within the normal daily range for this amino acid. Brain methionine and SAM were also significantly elevated.

Feeding study. To determine whether the changes in serum amino acids caused by the consumption of individual meals could, by modifying serum amino acid concentrations, elevate brain SAM concentrations, fasted animals weighing 250–350 g were given access to either no food (i.e. controls), 25 g of the protein-free diet, or 25 g of a 40% casein diet (Table 2) at 10 p.m. Three hours later the animals were killed. Brain samples were analysed for methionine and SAM, and serum for methionine and tyrosine. One animal in each of the experimental groups consumed less than 7 g

TABLE 2. COMPOSITION OF EXPERIMENTAL DIETS

Ingredient	Amount in diet (g) Protein-free	40% Casein
Casein	0	400
Dextrose	270	133
Sucrose	221	95
Dextrin	270	133
Mazola	150	150
Rogers–Harpers salt mix*	40	40
Vitamin mix†	10	10
Choline‡	2	2
Agar	35	35
Water	1000	1000

* ROGERS & HARPER (1965).
† WURTMAN *et al.* (1968).
‡ Added in water, 1 g/5 ml.

TABLE 1. EFFECTS OF INJECTING VARIOUS DOSES OF METHIONINE ON BRAIN AND SERUM METHIONINE AND BRAIN SAM

Dose (mg/kg)	(Per cent of control) Serum methionine	Brain methionine	Brain SAM
0 (Control)	100 ± 3	100 ± 5	100 ± 5
10	105 ± 10	108 ± 4	95 ± 4
30	138 ± 10*	177 ± 16‡	115 ± 2*
100	452 ± 47†	556 ± 66‡	121 ± 7*

Groups of six animals received i.p. injections of L-methionine and were killed 2 h later. Significant differences with respect to control values are shown by: * $P < 0{\cdot}05$; † $P < 0{\cdot}01$; ‡ $P < 0{\cdot}001$.

TABLE 3. EFFECTS OF CONSUMING PARTICULAR MEALS ON METHIONINE METABOLISM

Group	Serum methionine (nmol/ml)	Brain methionine (nmol/g)	Brain SAM (μg/g)	Serum tyrosine (nmol/ml)	R*
A	68·4 ± 2·1	32·9 ± 0·7	9·9 ± 0·2	97·2 ± 5·2	0·71 ± 0·04
B	48·0 ± 1·7‖	50·1 ± 2·1‖	11·0 ± 0·2†	44·8 ± 1·4‖	1·08 ± 0·11§
C	57·1 ± 1·0‡	49·0 ± 1·9‖	11·1 ± 0·3†	66·3 ± 1·9§	0·91 ± 0·05‡

Groups of six animals were fasted for 24 h starting at 10 p.m. and were then given: (A) no food (control); (B) 25 g of a protein-free diet; or (C) 25 g of a 40% casein diet. They were killed 3 h later. Data are given as means ± S.E.M.

* The ratio of serum methionine to serum tyrosine.

† $P < 0{\cdot}05$ relative to group A.

‡ $P < 0{\cdot}02$ relative to group A.

§ $P < 0{\cdot}01$ relative to group A.

‖ $P < 0{\cdot}001$ relative to group A.

and was excluded. All of the others ate 13 g or more.

Serum methionine concentrations compared to control were significantly decreased after either diet, especially the protein-free diet. Brain methionine, however, was significantly increased in both cases, as was brain SAM (Table 3). In a subsequent study, serum methionine did not fall in the protein-fed group; however, the changes in brain were similar to those described in Table 3. Serum tyrosine concentrations were significantly decreased in both of the fed groups; the calculated ratio of serum methionine to tyrosine was significantly increased relative to control in both cases, and in the subsequent study.

DISCUSSION

These studies suggest that brain methionine concentrations do vary in response to physiologic changes in serum amino acid patterns: they rise following treatments that increase the ratio of the serum methionine concentration to the sum of the neutral amino acids that compete with methionine for entry into the brain. Our data also indicate that physiological increases in brain methionine can cause significant elevations in brain SAM concentrations, probably by increasing the rate at which SAM is synthesized.

Amino acids are transported from the blood into brain slices by a saturable, energy-dependent process. Amino acids of similar structure compete for entry (BLASBERG & LAJTHA, 1965, 1966); methionine belongs to the group of large neutral amino acids, along with phenylalanine, tyrosine, tryptophan, leucine, isoleucine and valine (BLASBERG & LAJTHA, 1966). FERNSTROM & WURTMAN (1972) have shown that the effects of various diets on the brain tryptophan concentration are, in general, parallel to the changes that these diets produce in the ratio of serum tryptophan to the sum of serum phenylalanine, tyrosine, leucine, isoleucine and valine. This indicates that competition between neutral amino acids also occurs *in vivo* for transport into the brain. The ratio of plasma tryptophan to tyrosine provides almost as good a predictor of brain tryptophan levels as the ratio of tryptophan to the sum of the five neutral amino acids (FERNSTROM *et al.*, 1973).

The present data indicate that under physiological circumstances, brain methionine levels are also influenced by the competition between methionine and other neutral amino acids for transport into the brain. In the feeding study, the ratio of methionine to tyrosine in serum rose acutely following consumption of either the protein-free or the 40% casein diet; concomitantly, the brain methionine level was elevated, in spite of significant decreases in serum methionine *per se* (Table 3). The observations made in our rhythm studies also support the postulated relationship between brain methionine and plasma amino acid patterns. Thus, brain methionine concentration failed to exhibit a diurnal rhythm (Fig. 1b), nor did the calculated ratio of plasma methionine, measured here, to the sum of the six competing amino acids measured by FERNSTROM *et al.* (1971). However, serum methionine concentrations did exhibit a significant rhythm. The selective, several-fold elevation of serum methionine that followed its intraperitoneal injection also caused brain methionine to increase (Fig. 2, Table 1).

The present studies also show that brain SAM concentrations are sensitive to physiological increases in the availability of its amino acid precursor. SAM crosses only poorly from the blood into the brain, and its concentration in serum is very low; hence most of the SAM found in the brain must be synthesized within this tissue (BALDESSARINI & KOPIN, 1966) through the action of the enzyme ATP: l-methionine *S*-adenosyltransferase EC 2.4.2.13 (methionine adeno-

syltransferase; methionine activating enzyme) (AXELROD *et al.*, 1959). The K_M for this enzyme, $0{\cdot}9 \times 10^{-4}$ M (PAN & TARVER, 1967; MATTHYSSE *et al.*, 1972), is greater than mean brain methionine concentrations (Fig. 1). Thus the increase in brain SAM occuring after either diet was consumed (Table 3) or after methionine was injected (Table 1) very likely resulted from the concurrent increases in brain methionine concentrations, which increased the saturation of the methionine activating enzyme. That other processes besides altered SAM synthesis can influence the brain SAM concentration is suggested by the findings of our rhythm study, in which diurnal fluctuations were observed in brain SAM, but not in brain methionine. Since neither brain methionine level nor methionine adenosyltransferase activity exhibited parallel changes, it is probable that the brain SAM rhythm reflected cycles in the rates at which SAM was being utilized. In support of this hypothesis, it has been shown that drug-induced increases in SAM utilization (e.g. for the *O*-methylation of L-DOPA) can deplete brain SAM levels (WURTMAN *et al.*, 1970).

In other studies from this laboratory it has been shown that rat brain contains all of the enzymes needed to regenerate methionine from homocysteine, using serine as a source of methyl groups and folic acid derivatives as cofactors (ORDONEZ & WURTMAN, 1974*a*). When unusual demands are made on brain methyl groups by administering large doses of substrates for methylation reactions (e.g. L-DOPA), brain SAM concentrations fall, but brain methionine remains unaltered (ORDONEZ & WURTMAN, 1973*a*). L-DOPA treatment does deplete brain methionine when given to folate-deficient rats (ORDONEZ & WURTMAN, 1974*b*). Taken with our present studies, these observations indicate that brain methionine can derive from at least two sources, i.e. uptake from the circulation, via a carrier-mediated process which methionine shares with other neutral amino acids, and from *de novo* synthesis. Perhaps synthesis of the amino acid in brain becomes important under special circumstances, as when large quantities of homocysteine have been generated by the utilization of SAM for methylation reactions. In contrast, the process controlling methionine uptake from the blood would allow the levels of the amino acid in brain to respond to a variety of nutritional and other inputs that modify the plasma amino acid pattern.

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