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Folic Acid Deficiency and Methyl Group Metabolism in Rat Brain: Effects of L-Dopa

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Rats deficient in folic acid were found to have decreased concentrations of *S*-adenosylmethione in brain, kidney, and liver. They also showed decreased concentrations of methionine in serum, but not in brain. Administration of L-dopa (a methyl acceptor) in doses comparable to those used in the treatment of Parkinson's disease caused significant reductions in the concentrations of brain methionine in rats deficient in folic acid (45%, 45 min after administration), but failed to alter methionine concentrations in control animals. The changes in brain methionine brought about by L-dopa were not paralleled by similar changes in serum methionine, which decreased by only 20%. These observations suggest that de novo methyl group synthesis contributes significantly to the maintenance of brain methionine concentrations. The possibility is raised that the daily requirements for folic acid and for vitamin B_{12} may be increased in human patients treated chronically with large doses of L-dopa.

O-Methylation constitutes a major pathway for the metabolism of exogenous L-dopa in mammals (1). After rats received a single injection of the catechol amino acid in a dose comparable with that used in the treatment of Parkinson's disease (i.e., 30–100 mg/kg) (2), its biotransformation to 3-*O*-methyl dopa causes the concentrations of *S*-adenosylmethionine (SAM) in brain, kidney, and adrenals to fall markedly (3, 4); however, the concentrations of free methionine in brain and serum are not altered (4). Two injections of L-dopa at 45-min intervals reduce serum methionine concentrations by 40% without affecting brain methionine; only after four such injections do brain methionine concentrations become significantly depressed (by 30%; $P < 0.001$) (4). Since rat brain contains all of the enzymes needed to regenerate methyl groups from serine (5), and ultimately from glucose, we have suggested that its capacity to maintain methionine concentrations after doses of L-dopa that deplete brain SAM or serum methionine results from its ability to regenerate methionine from the homocysteine formed after the transmethylation of dopa and its catechol derivatives.

The three enzymes required for synthesizing the methyl groups of methionine from serine (serine transhydroxymethylase, [EC 2.1.2.1.], 5, 10-methylene tetrahydrofolate reductase [methylene reductase, EC 1.1.1.68.], and N5-methyltetrahydrofolate; homocysteine [cobalamin] methyltransferase [B_{12}-transmethylase]) utilize folic acid derivatives as cofactors. We have examined the effect of chronic folate deficiency on the metabolism of brain methyl groups after the administration of L-dopa.

MATERIALS AND METHODS

Male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) initially weighing 100 g were exposed to light (Vita-Lite, Duro-Test Corp., North Bergen, NJ) from 9 AM to 9 PM daily and were given free access for 2 months to water and to one of the following diets: (a) Rat Folic

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Deficient Diet (Nutritional Biochemicals) containing 2% succinyl sulfathiazole (folic-deficient group); (b) diet a supplemented with folic acid (5 mg/kg diet; folic-supplemented control group); or (c) Purina Laboratory Rat Chow (normal chow control group). Animals were then either decapitated or treated with L-dopa (100 mg/kg body weight) or its diluent (0.05 N HCl) 45 or 90 min prior to decapitation.

Blood collected from the cervical wound was assayed in a commercial laboratory (Clin-Chem Laboratories, Boston, MA) for folic acid by the growth response of *Lactobacillus casei*, and for vitamin B_{12} concentration by competitive binding to intrinsic factor. Brains, livers, and kidneys were removed and frozen on dry ice until they could be homogenized in 10% TCA/0.05 N HCl. The amount of SAM in tissues was measured by the method of Baldessarini and Kopin (6), and methionine was measured in brain and serum by the tRNA binding assay previously described (4). The concentrations of SAM and methionine are expressed as percentage of chow-fed untreated animals; absolute concentrations have been previously reported (4). Tissues to be assayed for enzymes were homogenized in phosphate buffer (0.1 M, pH 7.5) containing 10^{-3} M reduced glutathione; after centrifugation for 30 min at 34,000*g*, the supernatant fluids were dialyzed against the same buffer for 3 hr prior to assay. Methylene reductase activity was measured by the method of Kutzbach and Stokstad (7); B_{12}-transmethylase activity was assayed by the method of Taylor and Weissbach (8). Protein concentrations were measured in the dialyzed extracts by the method of Lowry *et al.* (9). Enzyme activities are expressed as nmoles of product formed/mg protein/min.

RESULTS

Metabolic changes in the folic-deficient rat. The rate at which body weight increased in rats given folic-deficient or folic-supplemented diets was significantly lower than among animals receiving the standard rat chow (Table I). Brain weights did not differ significantly among the three experimental groups; kidney weights, in confirmation of previous findings in animals with combined folic acid and vitamin B_{12} deficiency (10), were significantly elevated among the folic-deficient animals (Table I). Serum folate concentrations were markedly depressed in animals consuming the folic-deficient diet; vitamin B_{12} concentrations were similar in animals consuming the folate-deficient and folate-supplemented diets, but only about 60% of those found in rats eating the chow diet (Table I).

Previous studies have suggested that the enzymes methylene reductase or B_{12}-transmethylase might be rate-limiting in the *de novo* synthesis of brain methyl groups (5). The specific activities of brain methylene reductase assayed *in vitro* were the same in all three experimental groups (Table II); however, brain B_{12}-transmethylase was about 28% lower among animals consuming the folate-deficient diet than among chow-fed control rats, and was slightly lower than among animals eating the folic-supplemented diet (Table III).

TABLE I

ORGAN WEIGHTS, SERUM FOLIC ACID, AND VITAMIN B_{12} LEVELS OF RATS ON DIETS CONTAINING DIFFERENT AMOUNTS OF FOLIC ACID[a]

	Folic deficient	Folic supplemented	Chow fed
Body weight (g)	334.5 ± 10.1**	362.3 ± 12.9*	405.8 ± 7.4
Brain weight (g)	1.972 ± 0.019	2.016 ± 0.021	2.003 ± 0.016
Kidney weight (g)	1.713 ± 0.074**†	1.497 ± 0.048*	1.363 ± 0.030
Serum folates (ng/ml)	19.7	200	138.7
Serum vitamin B_{12}	276	271	459

[a] For the organ measurements, tissues from 18 animals maintained for 8½ weeks on either folic-deficient, folic-supplemented, or normal laboratory diets, were used. For the serum determinations, three samples each were obtained by pooling equal volumes of serum from two animals and assayed. Folate levels were measured by the growth response of *L. casei* and vitamin B_{12} levels by competitive binding to B_{12} intrinsic factor. Values reported are means of these determinations. * $P < 0.05$ differs from chow-fed controls. ** $P < 0.001$ differs from chow-fed controls. † $P < 0.01$ differs from folic-supplemented control animals.

TABLE II

SPECIFIC ACTIVITY OF METHYLENE REDUCTASE IN TISSUES OF FOLIC-DEFICIENT ANIMALS[a]

	Activity (nmoles × mg^{-1} × min^{-1})		
	Brain	Liver	Kidney
Folic acid deficient	0.023 ± 0.001	0.087 ± 0.004*†	0.164 ± 0.015
Folic acid supplemented	0.023 ± 0.003	0.109 ± 0.006	0.174 ± 0.008
Chow fed	0.023 ± 0.001	0.099 ± 0.003	0.154 ± 0.008

[a] Brain, liver, and kidneys from animals maintained for 8½ weeks on either folic-deficient, folic-supplemented, or normal rat diets were treated as described in Materials and Methods. The values are means ± SEM from six separate determinations for each group. * $P < 0.01$ from chow-fed controls. † $P < 0.01$ from folic-supplemented controls.

TABLE III

SPECIFIC ACTIVITY OF B_{12}-TRANSMETHYLASE IN TISSUES OF FOLIC-DEFICIENT ANIMALS[a]

	Activity (nmoles × mg^{-1} × min^{-1})		
	Brain	Liver	Kidney
Folic acid deficient	0.032 ± 0.001**†	0.019 ± 0.002**	0.099 ± 0.004**††
Folic acid supplemented	0.035 ± 0.001**	0.019 ± 0.002**	0.140 ± 0.007*
Controls	0.044 ± 0.001	0.040 ± 0.003	0.176 ± 0.009

[a] Brain, liver, and kidneys from groups of six animals maintained for 8½ weeks on either folic-deficient, folic-supplemented, or normal rat diets were treated as described in Materials and Methods. The values shown represent means ± SEM. * $P < 0.02$ differs from chow-fed controls. ** $P < 0.001$ differs from chow-fed controls. † $P < 0.02$ differs from folic-supplemented group. †† $P < 0.001$ differs from folic-supplemented group.

Livers from folic-deficient animals showed lower methylene reductase activities than similar tissues from control groups (Table II), while B_{12}-transmethylase activity was the same in folic-deficient and folic-supplemented animals but lower than in chow-fed controls (Table III). Kidneys showed no differences in methylene reductase activities (Table II), but the activity of B_{12}-transmethylase in this tissue was lower in folic-deficient animals than in either control (Table III).

Effect of L-*dopa on brain SAM and methionine concentrations in the folic-deficient rat.* The folic-deficient animals showed significant decreases in brain SAM (Fig. 1) and no changes in the tissue concentrations of methionine (Fig. 2), when compared with either group of controls. The administration of L-dopa led to similar decreases after 45 min in brain SAM concentrations among all three groups studied; at 90 min SAM concentrations were slightly elevated in folic-deficient animals, while in both control groups the

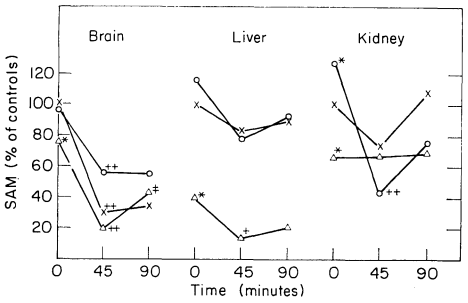

FIG. 1. SAM concentrations in folic-deficient rats at different times after the administration of L-dopa (A). SAM was measured in groups of six animals that were on folic-deficient (△), folic-supplemented (○), or normal chow (×) diets at 0, 45, or 90 min after a single administration of L-dopa (100 mg/kg). The results are expressed as percentage of the chow-fed group at 0 min. Absolute values for this group were: brain, 10.2 ± 0.8 μg/g; liver, 38.7 ± 3.1 μg/g; and kidney, 18.7 ± 1.9 μg/g. * $P < 0.001$ from chow-fed group at 0 min. † $P < 0.05$ from own group at 0 min. †† $P < 0.001$ from own group at 0 min. ‡ $P < 0.001$ from own group at 45 min.

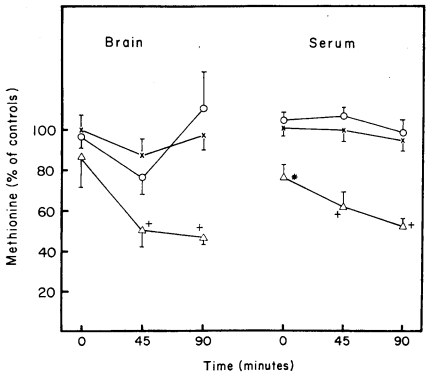

FIG. 2. Methionine concentrations of folic-deficient rats at different times after administration of L-dopa (A). Methionine was measured in brain and serum of groups of six animals maintained on folic-deficient (△), folic-supplemented (○), or control (×) diets at 0, 45, or 90 min after a single administration of L-dopa (100 mg/kg). The results are expressed as percentage of the control group as 0 min. Absolute values were: brain, 35.6 ± 1.7 nmole/g; serum, 79.7 ± 5.0 nmole/ml. * $P < 0.02$ from control groups at 0 time. † $P < 0.01$ from own group at 0 time.

concentrations of the metabolite remained at the low levels found 45 min after administration of the drug (Fig. 1).

The administration of L-dopa caused brain methionine concentrations to fall significantly among folic-deficient animals (Fig. 2). No significant changes in methionine concentrations in brain were observed after the administration of L-dopa in either folic-supplemented or chow-fed animals.

SAM concentrations in the livers and kidneys of folic-deficient animals were markedly lower than in folic-supplemented or chow-fed control groups (Fig. 1). Similarly, methionine concentrations in serum of folic-deficient animals were significantly lower than in the other groups of animals studied (Fig. 2). Among folic-deficient but not control animals, the administration of L-dopa caused liver SAM and serum methionine concentrations to decline significantly from their zero time values (Figs. 1–2). L-Dopa produced no changes in SAM concentrations in kidneys of folic-deficient animals (Fig. 1), but depressed renal SAM in both control groups.

DISCUSSION

Our observations indicate that the brain is capable of the de novo synthesis of methyl groups; this folic-mediated system normally allows brain methionine concentrations to remain fairly constant under conditions of increased transmethylation. Methionine concentrations were not depressed basally in brains of animals maintained for 2 months on a folic-deficient diet (Fig. 2); however, L-dopa, which causes increased transmethylation in the tissue (1), profoundly decreased methionine concentrations in folic-deficient (Fig. 2) but not in control animals.

The effects of folic deficiency on serum methionine concentrations both basally and after L-dopa treatment differ from its effects on brain methionine. Untreated animals deficient in folic acid showed significantly lower serum methionine concentrations than animals from either control group; L-dopa further decreased these concentrations by only 20% 45 min after administration (in contrast to brain, where the decrease was 42%). L-Dopa administration had no effect on serum methionine concentrations in control rats (Fig. 2).

Brains of untreated animals maintained for 2 months on the folic-deficient diet had lower B_{12}-transmethylase activity (Table III) and lower SAM concentrations (Fig. 1) than brains of either folic-supplemented or chow-fed control groups. The depressed activity of brain B_{12}-transmethylase in folic-deficient animals probably reflects lower vitamin B_{12} levels in this tissue, as suggested by lower serum B_{12} values in these animals (Table I). This could also explain the depressed activities of B_{12}-transmethylase in brain, liver, and kidney of folic-deficient and folic-supplemented animals when compared to normal chow-fed controls (Table III). Low levels of B_{12}-transmethylase activity have been reported to be associated with vitamin B_{12} deficiency (11). The decreased levels of the vitamin in serum of folic-deficient and folic-supplemented groups could be explained by inadequate amounts of B_{12} in the rat folic-deficient diet. They could also represent an increased vitamin requirement caused by destruction of the gut flora by succinyl sulfathizaole, as the gut bacteria normally provides some of the vitamin B_{12} utilized by the animals.

Apparently the lower activity of B_{12}-transmethylase in folic-deficient animals

when compared to chow-fed ones (Table III) is not responsible for the decreased capacity of the brain to maintain constant methionine levels after L-dopa (Fig. 2); brains of folic-supplemented controls that were able to maintain methionine concentrations after L-dopa also showed decreased B_{12}-transmethylase activities.

Similarly, alterations in methylene reductase activity cannot be the cause for the observed decrease in methionine concentrations in folic-deficient animals, since little or no change in the activity of this enzyme was observed in the brain and other tissues studied (Table II), confirming earlier reports by Stokstad (12).

As the folate moieties involved in the process are actually substrates in the enzymatic reactions leading to the de novo synthesis of the methyl groups of methionine (they carry the C-1 unit through every step in the entire process), probably the unavailability of folate derivatives per se is causing the observed inefficacy of the brain system to maintain constant methionine concentrations during the increased transmethylation of L-dopa.

Furthermore, our results show that alterations in methyl group metabolism occur in organs other than brain during folic acid deficiency. Untreated folic-deficient animals show lower than control SAM concentrations in liver and kidney (Fig. 1); serum methionine concentrations are decreased after administration of L-dopa, but no changes in serum methionine concentrations are observed in folic-supplemented or normal chow-fed animals (Fig. 2). The altered metabolism of methionine (a sulfur amino acid) in folic acid-deficient animals probably does not occur under vitamin B_{12} deficiency, as, to date, no abnormalities in sulfur amino acid metabolism have been found during deficiency of this vitamin (13). This suggests a more critical role for folic acid derivatives in the metabolism of sulfur amino acids than previously thought.

The present results suggest that during chronic L-dopa administration to human patients, the excessive utilization of the de novo pathway of methyl group synthesis could increase nutritional requirements for folic acid and vitamin B_{12}.

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