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The effect of L-dopa administration and folate deficiency on plasma homocysteine concentrations in rats

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O-Methylation of the anti-Parkinson's disease drug L-dopa leads to significant decreases of S-adenosylmethionine and significant increases of S-adenosylhomocysteine concentrations in tissues. Based on these observations, we hypothesized that L-dopa administration would also lead to increased production of homocysteine and hyperhomocysteinemia. This hypothesis was tested in two separate experiments. In experiment 1, control and folate-deficient male rats were injected intraperitoneally with 100 mg of L-dopa per kilogram body weight. After 1 hr, blood was collected and analyzed for homocysteine. Plasma homocysteine concentration was significantly higher in the rats treated with L-dopa than in the rats treated with vehicle alone. Furthermore, the apparent increase of plasma homocysteine due to L-dopa was greater in the folate-deficient rats than in the replete controls, suggesting a significant interaction between L-dopa administration and folate deficiency on plasma homocysteine concentration. In experiment 2, nondeficient female rats were injected intraperitoneally with 100 mg of L-dopa per kilogram of body weight for 0, 1, or 17 consecutive days (one injection per day). Blood was collected 1 hr after the last dose and analyzed for homocysteine. Plasma homocysteine concentration was significantly higher in the rats treated for 17 days than in the nontreated controls, indicating that the effect of L-dopa persisted with chronic administration. However, plasma homocysteine concentration was significantly higher in the rats treated with L-dopa for only 1 day than in those treated for 17 days, suggesting that there is some attenuation of the effect of L-dopa with chronic administration. Measurements of S-adenosylmethionine and S-adenosylhomocysteine in brain and liver were consistent with the hypothesis that the hyperhomocysteinemia was a consequence of significant O-methylation. (J. Nutr. Biochem. 8:634–640, 1997) © *Elsevier Science Inc. 1997*

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Introduction

L-3,4-dihydroxyphenylalanine (L-dopa) is generally recognized as the most beneficial drug treatment for the neurodegenerative movement disorder Parkinson's disease. It is effective because it crosses the blood–brain barrier and is decarboxylated to form the neurotransmitter dopamine (*Figure 1*), deficits of which in the nigrostriatal region of the brain are the primary concern in this disorder. Only a small percentage of an exogenous dose of L-dopa is actually converted to dopamine in the brain, however.[1–5] The majority is either decarboxylated in peripheral tissues to produce dopamine, which does not cross the blood–brain barrier, or is *O*-methylated in both peripheral and central tissues to produce 3-*O*-methyldopa (3-methoxy-4-hydroxyphenylalanine), a metabolite that does not serve as a biochemical precursor of dopamine.[6–8] Therefore, to effectively raise brain dopamine levels, a large amount of L-dopa must be administered, often as much as several grams in an oral dose. This is the case even when the L-dopa is coadministered with a peripheral L-dopa decarboxylase

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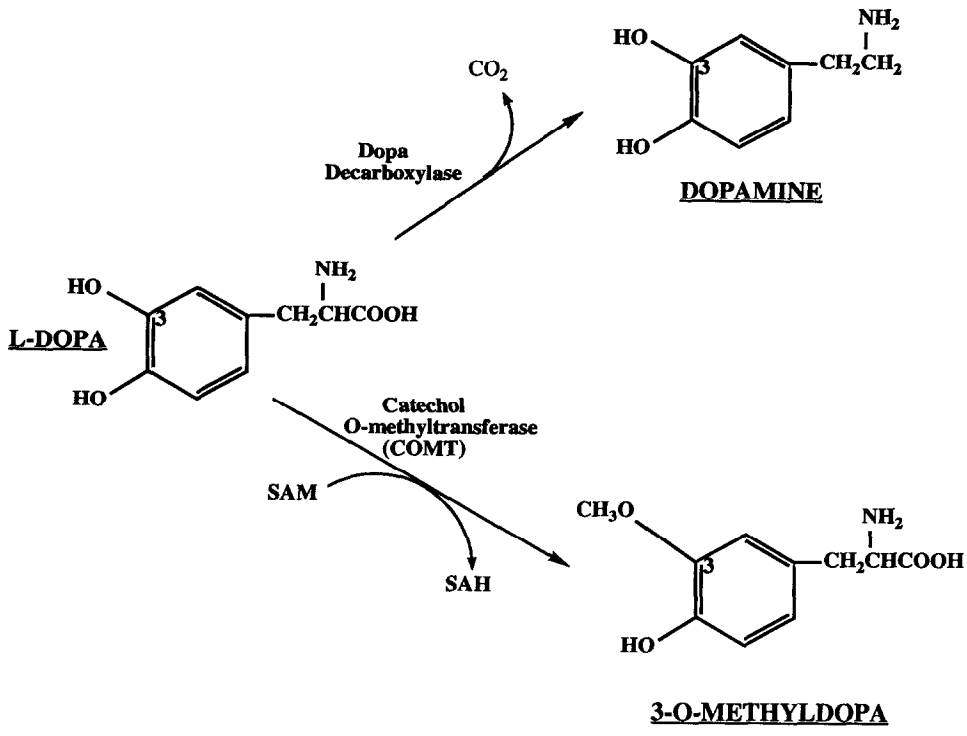

Figure 1 L-dopa metabolism. Abbreviations: SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine.

(E.C. 4.1.1.26) inhibitor (carbidopa, benserazide), as is the present standard of care.[9]

It has been recognized that such large doses of L-dopa can significantly affect sulfur amino acid metabolite concentrations. In the *O*-methylation reaction, the enzyme catechol *O*-methyltransferase (COMT) (E.C. 2.1.1.6) catalyzes the addition of a methyl group to the 3-hydroxyl position of L-dopa, with *S*-adenosylmethionine (SAM) serving as the methyl donor (*Figure 1*). In the process, the demethylated metabolite *S*-adenosylhomocysteine (SAH) is produced as a byproduct. Several studies in rats have established that there is a decrease in brain SAM concentration and an increase in brain SAH concentration after one dose of L-dopa[1,10–18] and a decrease in hepatic SAM concentration after repeated doses.[1,10,11,13,18] In humans, L-dopa has been associated with decreased SAM concentrations in blood[19] and cerebrospinal fluid.[20]

Based on these findings, we hypothesized that another consequence of high-dose L-dopa administration might be hyperhomocysteinemia. SAH produced during *O*-methylation reactions is hydrolyzed to form homocysteine and adenosine (*Figure 2*). Consequently, when L-dopa is administered in large quantities, a significant increase in homocysteine production is expected. This increase in production might overburden metabolic pathways and lead to the export of unmetabolized homocysteine into the blood, causing hyperhomocysteinemia. Moreover, the resulting hyperhomocysteinemia might be accentuated if L-dopa administration is superimposed on a condition known to impair homocysteine metabolism, such as an enzyme defect or B vitamin deficiency (*Figure 2*). The present study was therefore undertaken to determine: 1) whether acute L-dopa administration is associated with hyperhomocysteinemia, 2) whether L-dopa-induced hyperhomocysteinemia is accentuated when homocysteine metabolism is impaired by folate deficiency, and 3) whether hyperhomocysteinemia persists with chronic L-dopa administration. In addition, SAM and SAH concentrations in liver and brain were determined to confirm the previously observed effect of L-dopa on these sulfur amino acid metabolites.

Methods and materials

Animals and diets

All experimental protocols were approved by the Institutional Animal Care and Use Committee of Tufts University. In experiment 1, weanling male Sprague–Dawley rats were obtained from Charles River Breeding Laboratories (Wilmington, MA) and housed in stainless-steel cages at 22–24°C. Light was cycled 12 hr on/12 hr off and water was available ad libitum. Upon arrival, the rats were acclimated for 4 days, during which time they were fed a vitamin-replete amino acid–defined diet.[21] The rats were then paired by weight and the pairs randomly assigned to receive the same amino acid–defined diet containing 1% succinylsulfathiazole and 0 or 8 mg folate per kilogram of diet. The rats were fed this diet ad libitum for 4 weeks. In experiment 2, female Sprague–Dawley retired breeder rats (Charles River Breeding Laboratories, Wilmington, MA) weighing approximately 300 g were housed and allowed access to water as described above. These rats were fed a standard rat chow diet (Agway, Watham, MA) replete with all essential nutrients, including folate.

L-dopa administration and sample preparation

In experiment 1, after 4 weeks of feeding, the rats were fasted overnight and then administered intraperitoneally (i.p.) either 100 mg of L-dopa per kilogram body weight (10 mg of L-dopa per milliliter of 0.05 N HCl) or an appropriate volume of 0.9% saline. In experiment 2, rats received i.p. injections of L-dopa (same amount and concentration as in experiment 1) for 0, 1, or 17

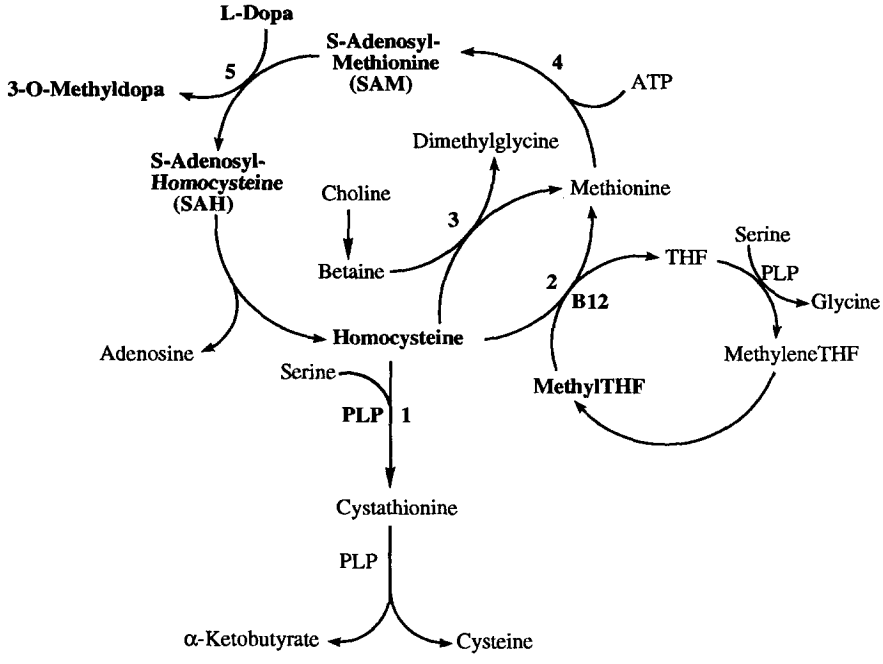

Figure 2 Homocysteine synthesis and metabolism. Hyperhomocysteinemia can result from impairments of homocysteine metabolism caused by enzyme defects and B vitamin deficiencies, or, as indicated by the present study, by increased synthesis of homocysteine as a result of excess methylation. Enzymes: 1, cystathionine β-synthase; 2, 5-methyltetrahydrofolate homocysteine methyltransferase (E.C. 2.1.1.13); 3, betaine-homocysteine methyltransferase; 4, methionine adenosyltransferase; 5, catechol-O-methyltransferase. Abbreviations: THF, tetrahydrofolate; PLP, pyridoxal-5′-phosphate; B12, vitamin B_{12}.

consecutive days (one injection per day). All rats were killed by CO_2 asphyxiation 1 hr after the last injection, and blood from each rat was immediately collected from the caudal vena cava into a vacutainer tube containing EDTA and centrifuged at 500 × *g* for 10 min at 4°C. The plasma supernatant then was collected and stored at −70°C until analysis. In addition, the livers and brains from each rat were excised, frozen immediately in liquid nitrogen, and stored at −70°C until analysis. L-dopa was obtained from Sigma Chemical Co. (St. Louis, MO).

Analytical methods

Total plasma homocysteine concentration was determined using high performance liquid chromatography (HPLC) with flourimetric detection by the method of Vester and Rasmussen.[22] Folate concentration in plasma was determined by microtiter plate assay using *Lactobacillus casei*.[23,24] Plasma pyridoxal-5′-phosphate (PLP) concentration was determined by the tyrosine decarboxylase method of Camp et al.[25] with modifications.[26] Hepatic SAM and SAH concentrations were determined by HPLC with UV detection using the method of Fell et al.[27] with modifications.[26]

Statistical analysis

Differences in folate, homocysteine, SAM, and SAH concentrations between experimental groups were detected using one and two-factor analyses of variance followed by Tukey's honestly significant difference (HSD) test.[28] Because the within-group variabilities of the homocysteine measurements were seen to increase with each experimental group's mean response, a square root transformation was applied to the data prior to the analysis. Square root was used because it was the transformation that best equalized the variability within each group. To determine whether the effect of L-dopa on plasma homocysteine, as measured in the original scale, was greater in the folate-deficient rats than in the folate-replete controls, we compared the difference between the effects of L-dopa in the deficient and replete rats to the standard error based on large sample theory.[28] The statistics were performed using Systat 5 for the Macintosh (Systat, Evanston, IL) and SPSS statistical software (SPSS Inc., Chicago, IL). Statistical significance for all data was defined as $P \leq 0.05$.

Results

Experiment 1: Effect of acute L-dopa administration in folate-deficient and replete male rats

Table 1 shows the folate status of the rats fed the folate-replete and folate-deficient diets for 4 weeks. Total folate concentrations in plasma, liver, and brain were significantly lower in the rats fed the folate-deficient diet ($P \leq 0.001$). Folate concentrations in these three compartments were not affected by L-dopa administration.

Figure 3 shows the effect of acute L-dopa administration on total plasma homocysteine in folate-replete and folate-deficient rats. In the folate-replete rats, plasma homocysteine concentration was approximately 2-fold higher in those treated with L-dopa than in those treated with vehicle alone (mean ± SD: 16.9 ± 6.4 vs. 8.0 ± 5.5 μmol/L; $P = 0.05$). For the folate-deficient rats, plasma homocysteine concentration also was higher in the L-dopa-treated rats than

Table 1 Plasma, hepatic, and brain total folate concentrations in folate-replete and folate-deficient male rats administered a single acute dose of L-dopa or vehicle*

Experimental group	Folate concentrations		
	Plasma (ng/mL)	Liver (μg/g)	Brain (μg/g)
Folate replete (n = 6)			
Without L-dopa	66.6 ± 21.6	6.92 ± 1.43	0.49 ± 0.09
With L-dopa	67.8 ± 8.2	7.01 ± 1.55	0.52 ± 0.06
Folate deficient (n = 6 or 7)			
Without L-dopa	0.88 ± 0.27†	0.42 ± 0.16†	0.19 ± 0.10†
With L-dopa	0.81 ± 0.47†	0.59 ± 0.19†	0.24 ± 0.06†

*Means ± SD.
†Significantly different from folate replete: $P \leq 0.001$.

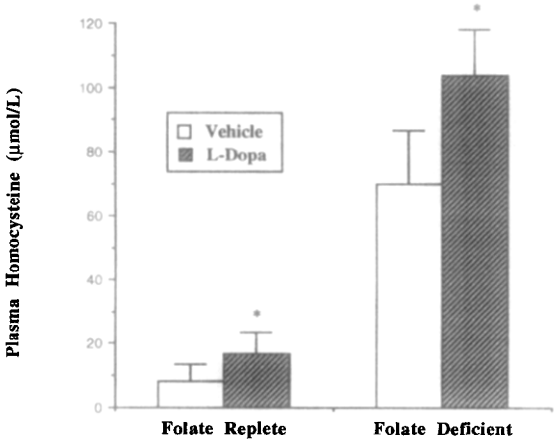

Figure 3 Effect of a single acute dose of L-dopa on total plasma homocysteine concentration in folate-replete and folate-deficient male rats. Plasma homocysteine concentration was significantly higher in the rats treated with L-dopa than in those treated with vehicle alone. In addition, the effect of L-dopa was greater in the folate-deficient rats than in the folate-replete controls ($P = 0.008$). Means ± SD; $n = 6$ or 7. *Significantly different from vehicle-treated controls, $P \leq 0.05$.

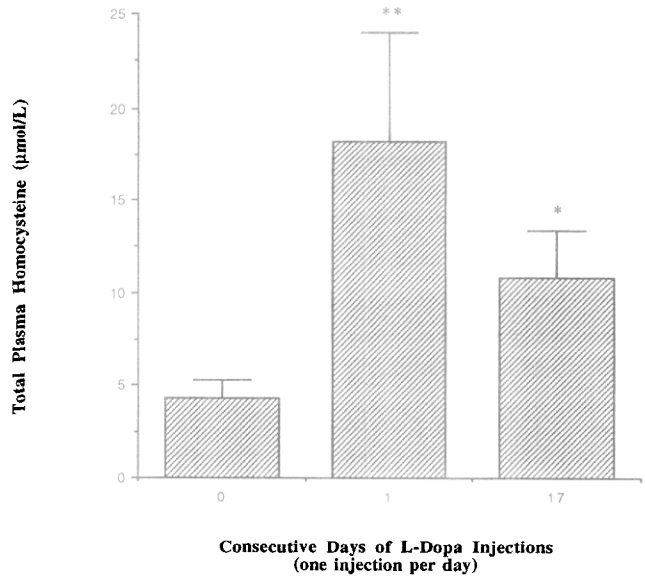

Figure 4 Effects of acute and chronic L-dopa administration on total plasma homocysteine concentration in nondeficient female rats. Plasma homocysteine concentration was significantly higher in the rats treated with L-dopa for 17 consecutive days (one injection per day) than in the nontreated controls. Plasma homocysteine in the once-treated rats was significantly higher than in both the chronically treated rats and the nontreated controls. Means ± SD; $n = 4$. *Significantly different from nontreated controls, $P = 0.015$. **Significantly different from chronically treated rats and nontreated controls, $P \leq 0.047$.

those treated with vehicle (103.7 ± 14.2 vs. 70.2 ± 16.9 μmol/L; $P = 0.004$). In addition, there was a statistically significant interaction between L-dopa administration and folate status on the plasma homocysteine concentration; the apparent increase in plasma homocysteine due to L-dopa was greater in the folate-deficient rats than in the folate-replete rats (apparent δ of 33.5 and 8.9 μmol/L, respectively; $P = 0.008$).

The effect of acute L-dopa administration on hepatic and brain SAM and SAH concentrations is presented in *Table 2*. In the folate-replete rats, no difference in hepatic SAM concentration was observed between those treated with L-dopa and those treated with vehicle. Brain SAM concentration, however, was significantly lower in the L-dopa-treated rats ($P < 0.001$). SAH concentrations in both the liver and brain were significantly higher in the L-dopa-treated rats ($P \leq 0.002$). In the folate-deficient rats, L-dopa treatment had the same general effect on hepatic and brain SAM and SAH concentrations as in the folate-replete rats, except that no difference in hepatic SAH concentration was observed.

Experiment 2: Effects of acute and chronic L-dopa administration in nondeficient female rats

The effects of both acute and chronic L-dopa administration on total plasma homocysteine concentration in nondeficient female rats is presented in *Figure 4*. Mean plasma homocysteine was 2.5-fold higher in those rats treated chronically with L-dopa for 17 consecutive days (one injection per day) than in controls who received no L-dopa injections (10.8 ± 2.6 vs. 4.3 ± 1.0 μmol/L; $P = 0.015$). In rats treated with only one acute dose of L-dopa, mean plasma homocysteine (18.2 ± 5.8 μmol/L) was significantly higher than for both the chronically treated rats ($P = 0.047$) and the nontreated controls ($P < 0.001$).

The effect of chronic L-dopa administration on hepatic and brain SAM and SAH concentrations is presented in

Table 2 Effect of a single acute dose of L-dopa on hepatic and brain SAM and SAH concentrations in folate-replete and folate-deficient male rats*

Experimental group	Liver		Brain	
	SAM (nmol/g)	SAH (nmol/g)	SAM (nmol/g)	SAH (nmol/g)
Folate replete ($n = 6$)				
Without L-dopa	30.8 ± 6.6	10.5 ± 1.0	11.5 ± 0.6	0.5 ± 0.1
With L-dopa	32.6 ± 12.4	17.5 ± 4.1†	5.0 ± 0.4†	1.7 ± 0.3†
Folate deficient ($n = 6$ or 7)				
Without L-dopa	9.2 ± 1.3	51.8 ± 6.2	10.3 ± 0.7	0.8 ± 0.2
With L-dopa	8.1 ± 2.3	45.6 ± 7.5	5.4 ± 0.4†	3.7 ± 0.5†

*Means ± SD.
†Significantly different from vehicle-treated controls: $P \leq 0.002$.

Table 3 Effects of acute and chronic L-dopa administration on hepatic and brain SAM and SAH concentrations in nondeficient female rats*

Consecutive days of L-dopa injections (one injection per day)	Liver		Brain	
	SAM (nmol/g)	SAH (nmol/g)	SAM (nmol/g)	SAH (nmol/g)
0	32.5 ± 4.1	11.8 ± 2.6	11.3 ± 0.3	0.6 ± 0.1
1	20.4 ± 7.9†	14.3 ± 1.4	6.0 ± 0.6†	1.4 ± 0.2†
17	34.3 ± 4.2	14.6 ± 2.6	6.3 ± 0.4†	1.0 ± 0.2‡

*Means ± SD.
†Significantly different from non-L-dopa-treated controls: $P \leq 0.037$.
‡Significantly different from rats treated once with L-dopa: $P \leq 0.021$.

Table 3. Mean hepatic SAM concentration in rats treated with L-dopa for 17 consecutive days (one injection per day) was not significantly different from untreated controls. In contrast, mean hepatic SAM concentration in those rats treated with only one dose of L-dopa was significantly lower than in both the chronically treated rats ($P = 0.018$) and the nontreated controls ($P = 0.037$). Mean hepatic SAH concentration was not significantly different among the three groups. Mean brain SAM concentration was significantly lower in both chronically treated ($P < 0.001$) and once-treated rats ($P < 0.001$) than in the nontreated controls. Mean brain SAH concentration was significantly higher in the chronically treated rats than in the nontreated controls ($P = 0.009$). In the rats treated with only one dose of L-dopa, mean brain SAH concentration was significantly higher than both the chronically treated rats ($P = 0.021$) and the nontreated controls ($P < 0.001$).

Discussion

The primary findings of experiment 1 were as follows. First, the mean plasma homocysteine concentration was higher in male rats treated with a single acute dose of L-dopa than in those treated with vehicle alone. This was consistent with the central hypothesis that *O*-methylation of exogenously administered L-dopa leads to overproduction of homocysteine and consequent hyperhomocysteinemia. Second, the mean apparent increase of plasma homocysteine concentration caused by L-dopa was greater in the folate-deficient rats than in folate-replete controls. This indicated that the severity of L-dopa-induced hyperhomocysteinemia was dependent on the status of folate, a vitamin essential for the remethylation of homocysteine to form methionine. Therefore, the effect of L-dopa administration on plasma homocysteine concentration was more pronounced when superimposed on a condition of impaired homocysteine metabolism.

Support for the hypothesis that the hyperhomocysteinemia resulted from the *O*-methylation of L-dopa derives from the tissue measurements of SAM and SAH. For the male rats treated with a single acute dose of L-dopa, the results were consistent with previous reports[1,10–18]; the mean brain SAM concentration was significantly lower and the mean brain SAH concentration was significantly higher than in untreated controls. In contrast, the liver was more resistant to the effects of L-dopa; while the mean hepatic SAH concentration was significantly elevated, the mean hepatic SAM concentration was not different from controls. A possible interpretation of this finding is that the liver is more efficient than the brain in regenerating SAM concentrations after exposure to L-dopa. This may be related to tissue-specific differences in enzyme activities involved in the remethylation of homocysteine to methionine and the activation of methionine to form SAM. (This primarily refers to the fact that mammalian liver, unlike the brain, has an alternative pathway for conversion of homocysteine to methionine that utilizes the choline metabolite betaine as the methyl donor and the enzyme betaine-homocysteine methyltransferase [E.C. 2.1.1.5] [(see *Figure 2*)[29,30].) Nevertheless, the elevated hepatic SAH concentration in the L-dopa-treated rats indicates that *O*-methylation did occur in the liver, as it did in the brain.

The primary findings of experiment 2 were as follows. First, as for the male rats, the mean plasma homocysteine concentration was higher in the female rats treated with a single acute dose of L-dopa than in those treated with vehicle alone. Again, this was consistent with the central hypothesis that hyperhomocysteinemia results from the *O*-methylation of L-dopa. Second, the mean plasma homocysteine concentration in female rats treated for 17 consecutive days (one injection per day) was significantly higher than in untreated controls. Therefore, hyperhomocysteinemia persisted even during chronic L-dopa administration. However, the mean plasma homocysteine concentration in the rats treated for 17 days was significantly lower than in the rats treated for only 1 day. This suggested that there was an adaptation to chronic L-dopa administration that led to less homocysteine being exported from tissues into the blood.

As for the male rats, measurements of tissue SAM and SAH concentrations in the female rats were consistent with significant *O*-methylation of L-dopa. They also provided further evidence of an adaptive response to chronic L-dopa administration as cited above for plasma homocysteine. In the brain, both the rats treated with a single acute dose of L-dopa and those treated chronically for 17 consecutive days exhibited a significantly lower mean SAM concentration and a significantly higher mean SAH concentration than untreated controls, thus indicating that *O*-methylation had occurred in this tissue. The chronically treated rats, however, had a significantly lower brain SAH concentration than the once-treated rats, suggestive of an adaptation to the L-dopa. In the liver, the mean SAM concentration was significantly lower in the acutely treated female rats than in the untreated controls, while hepatic SAH was only insig-

nificantly higher. This, again, was indicative of *O*-methylation. In contrast, the chronically treated rats exhibited no differences in hepatic SAM and SAH concentrations from controls, suggesting again that there was an adaptation to the L-dopa. Thus, adaptation in the brain was toward restoring normal SAH levels, while adaptation in the liver was toward restoring SAM levels. The underlying mechanism(s) for these adaptations was not investigated in the present study, but may be related to changes in enzyme activities affecting sulfur amino acid metabolism. An example of such a phenomenon was found by Benson et al.,[31] who demonstrated that chronic L-dopa administration in mice caused a significant increase of methionine adenosyltransferase (E.C. 2.1.5.6) activity in the brain. This enzyme catalyzes the synthesis of SAM from methionine (*Figure 2*). Other enzymes involved in sulfur amino acid metabolism may be similarly affected.

The decrease in hepatic SAM concentration observed in the female rats treated with a single acute dose of L-dopa was in contrast with the male rats of experiment 1 in which no decrease of hepatic SAM was observed. An interpretation of this difference is that the male rats were more efficient than the females in regenerating hepatic SAM concentrations after exposure to L-dopa under the conditions of these experiments. It remains to be determined whether this was due to gender differences in the biochemistry of the liver, gender differences in body composition affecting the absorption of L-dopa from the intraperitoneal cavity (the female rats were observed to be smaller and to contain less intra-abdominal fat than the males), differences in age (the female rats were ~9 months old, while the males were only ~2 months old), or simply a statistical anomaly associated with a small sample size. Confirmation of this finding will require further study with larger sample sizes.

When considering the mechanism of L-dopa-induced hyperhomocysteinemia, another factor must be considered. L-dopa is known to form a Schiff base complex with PLP, the active form of vitamin B_6.[32,33] It has been suggested that this interaction produces a functional vitamin B_6 deficiency by decreasing the availability of PLP to the many enzymes that require it as a cofactor.[34,35] One of these enzymes is cystathionine β-synthase (E.C. 4.2.1.22), which initiates the catabolism of homocysteine through the transsulfuration pathway (see *Figure 2*). If cystathionine β-synthase activity is inhibited in this way, it could contribute to the hyperhomocysteinemia due to L-dopa administration by combining increased homocysteine synthesis with an impairment of homocysteine metabolism. This further suggests that vitamin B_6 deficiency might accentuate the effect of L-dopa on plasma homocysteine concentrations. It should be noted, though, that overt vitamin B_6 deficiency, i.e., a low plasma PLP concentration caused by L-dopa therapy, has not been reliably observed[36] and was not observed by us in our L-dopa-treated rats (unpublished data).

In recent years, elevated plasma homocysteine has received significant attention as an independent risk factor for cardiovascular, peripheral, and cerebrovascular diseases.[37] Consistent with the present study, Allain et al.[38] have observed an elevated mean plasma homocysteine concentration in a small population of Parkinson's disease patients on L-dopa therapy as compared to healthy, untreated controls, a finding we have recently confirmed (unpublished data). Further study is needed to determine whether such hyperhomocysteinemia has significant health implications for Parkinson's disease patients on chronic L-dopa therapy.

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