L-3,4-dihydroxyphenylalanine (levodopa) lowers central nervous system S-adenosylmethionine concentrations in humans

R Surtees, K Hyland

Abstract
To determine whether levodopa reduces the levels of S-adenosylmethionine in the human central nervous system, cerebrospinal fluid (CSF) concentrations of S-adenosylmethionine, methionine, 3-methoxytyrosine, levodopa and 5-methyltetrahydrofolate were measured in six children with dopamine deficiency before and after treatment. In four, the lack of dopamine was secondary to a reduction in concentration of levodopa and these were treated with levodopa together with a peripheral dopa-decarboxylase inhibitor. In the other two, levodopa in the central nervous system naturally accumulated due to a congenital deficiency of aromatic-L-amino acid decarboxylase and these were treated with pyridoxine (which in this condition lowers central levodoa concentrations). Raising levodopa concentrations in the central nervous system caused a fall in CSF S-adenosylmethionine concentration and a rise in CSF 3-methoxytyrosine concentration. No change was observed in CSF methionine concentration and in all patients CSF 5-methyltetrahydrofolate concentration was normal. With one exclusion there was a linear relationship between CSF S-adenosylmethionine and 3-methoxytyrosine concentrations. This is the first demonstration of such effects in humans and the implications upon levodopa therapy are discussed.

S-adenosylmethionine is a key intermediary in transmethylation reactions, polyamine synthesis and the formation of cysteine and taurine.[1] Factors that greatly affect its metabolism may therefore have serious consequences.

L-3,4-dihydroxyphenylalanine (levodopa) is used in humans in the treatment of Parkinsonism and dystonias,[2] and also in some inborn errors of metabolism where it bypasses the metabolic block.[3] The normal route of metabolism of levodopa is decarboxylation to form dopamine,[4] however, when given in pharmacological doses it is methylated by catechol-0-methyltransferase to form 3-methoxytyrosine in a reaction that uses S-adeno-

sylmethionine as a methyl-donor.[5] In 1970, Wurtman *et al* showed that administration of a single dose of levodopa to healthy rats (in an amount equivalent to that used to treat Parkinsonism in humans) caused a marked fall in brain S-adenosylmethionine concentrations for up to six hours.[6] It has also been shown that in rodents levodopa reduces the methylation of noradrenaline,[7] and that this can be reversed by the administration of S-adenosylmethionine.[8]

In this paper we demonstrate that elevation of levodopa concentrations in the central nervous system in children causes a marked reduction in the concentration of S-adenosylmethionine in the cerebrospinal fluid (CSF), and that this is accompanied by raised levels of 3-methoxytyrosine.

Patients and methods
Lumbar CSF was collected from three children with inborn errors of pteridine metabolism (two with dihydropteridine reductase deficiency and one with a tetrahydrobiopterin synthesis defect) and one child with akinetic mutism before and after initiation of therapy with levodopa. In addition, CSF was collected from two children with aromatic-L-amino-acid decarboxylase (L-AADC) deficiency before and after therapy with pyridoxine. Patient details are given in table 1. The patients with L-AADC deficiency will be the subject of a separte report; however, they showed marked accumulation of levodopa and 3-methoxytyrosine in the CSF which was partially responsive to treatment with pyridoxine.

The third millilitre of CSF was frozen at the bedside on solid carbon dioxide and stored at $-70°C$ until analysis. CSF S-adenosylmethionine was measured by high performance liquid chromatography (HPLC) with electrochemical detection.[9] CSF levodopa and 3-methoxytyrosine were measured by HPLC with fluorescence detection; the stationary phase was a 25×0.4 cm Apex 5 μm ODS column maintained at 35°C, the mobile phase 0·05 M sodium acetate buffer pH 4·75 containing 48 μM EDTA and 500 μl/l n-dibutylamine. Flow rate was 1·3 ml/min, and detection was by a Perkin-Elmer LS-3 fluorescence detector with the excitation wavelength and emission wavelengths set at 278 nm and 320 nm respectively. CSF 5-

Department of Child Health, Institute of Child Health, London
R Surtees
K Hyland

Correspondence to:
R Surtees, Department of Child Health, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, United Kingdom

Table 1 Patient details

Patient	Age (years)	Diagnosis	Levodopa (mg/kg/day)	Time between lumbar punctures (weeks)	Other drugs
1	2	DHPR deficiency	10	4	5-HTP CBD CHO-THF
2	0·6	DHPR deficiency	10	2	5-HTP CBD CHO-THF
3	0·5	Pterin synthesis defect	10	4	5-HTP CBD
4	14	Akinetic mutism	10	4	5-HTP CBD
5	0·5	L-AADC deficiency	NA	2	pyridoxine
6	0·5	L-AADC deficiency	NA	2	pyridoxine

DHPR = dihydropteridine reductase, L-AADC = aromatic-L-amino-acid decarboxylase, 5-HTP = 5-hydroxytryptophan, CBD = carbidopa, CHO-THF = 5-formyltetrahydrofolate, L-AADC = aromatic-L-amino acid decarboxylase, NA = not applicable.

Table 2 CSF S-adenosylmethionine (SAM), 3-methoxytyrosine (3MT), levodopa, 5-methyltetrahydrofolate (CH3THF) and methionine (MET) before and after treatment with levodopa or pyridoxine.

Patient	Therapy	SAM (nM)	3MT (ng/ml)	Levodopa (ng/ml)	CH3THF (ng/ml)	MET (μM)
1	none	205	<3	<2·5	48	5·6
	Levodopa	123	327	<2·5	66	2·6
2	none	308	<3	<2·5	32	2·1
	Levodopa	241	143	50·6	51	1·8
3	none	502	<3	<2·5	45	7·2
	Levodopa	303	804	139	61	30·5*
4	none	197	<3	<2·5	21	2·6
	Levodopa	129	339	<2·5	32	2·8
5	pyridoxine	173	174	33·9	34	9·2
	none	115	378	60·0	32	3·3
6	pyridoxine	217	210	40·8	35	2·3
	none	115	363	61·2	31	3·1
reference range		168–496	<3–10	<2·5	14–88	1·6–8·7

*This sample was blood-stained and also had raised concentrations of leucine, isoleucine, valine, tyrosine, phenylalanine and tryptophan.

methyltetrahydrofolate was measured by HPLC with electrochemical detection; the stationary phase was 25 × 0·4 cm Apex 5 μm ODS column maintained at 30°C, the mobile phase 0·05 M sodium acetate buffer, pH 4·6, containing 20% methanol, 48 μM EDTA and 35 mg/l dithioerythritol, flow rate 0·7 ml/min; detection was achieved using the first electrode of a Coulochem ESA 1101 electrochemical detector, with the analytical electrode at +0·05 V. CSF methionine was measured using 0-phthalaldehyde-2-mercaptoethanol derivatisation and HPLC-fluorimetry (after ref 10); the stationary phase was a 10 × 0·4 cm Apex 3 μm ODS column at room temperature, the mobile phase 0·1 M sodium acetate buffer, pH 5, containing 50% methanol, flow rate 1·3 ml/min, and detection by a Perkin-Elmer LS-4 fluorescence detector with excitation and emission wavelengths set at 340 and 450 nm respectively.

Reference ranges for the CSF metabolites were obtained by analysis of CSF taken from children with a wide variety of metabolic or neurological disease in whom disturbance of these pathways was not expected.

For statistical analysis all patients were treated as a single group. The patients investigated before starting levodopa were grouped with those with L-AADC deficiency receiving treatment with pyridoxine; and, vice versa, those taking levodopa were grouped with L-AADC deficient patients before treatment. Statistical analysis of the data pairs used the paired t-statistic and that of linear trends used analysis of variations. Mean differences are expressed as means (95th centile confidence limits).

Results

In all patients receiving levodopa there was a marked fall in CSF S-adenosylmethionine concentration and a rise in 3-methoxytyrosine concentration (table 2). Likewise, treatment of the patients with L-AADC deficiency with pyridoxine led to a fall in CSF levodopa and 3-methoxytyrosine concentrations that was associated with a rise in CSF S-adenosylmethionine. When all the results were considered together the mean fall in S-adenosyl-methionine concentrations was 96 (41–151) nM (p = 0·007), and the mean rise in 3-methoxy-
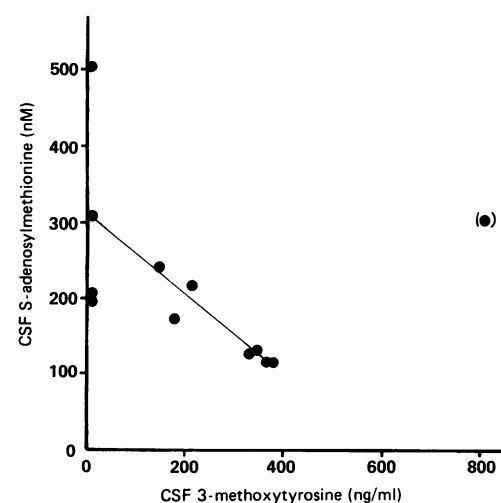

Figure 1 The relationship between CSF S-adenosylmethionine and 3-methoxytyrosine concentrations. The regression line was fitted by the method of least squares after omission of the one outlier (in brackets).
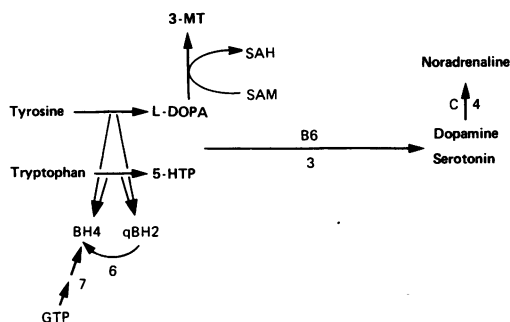
Figure 2 Synthesis of biogenic amines and methylation of levodopa. 1 = tyrosine hydroxylase, 2 = tryptophan hydroxylase, 3 = aromatic-L-amino acid decarboxylase, 4 = dopamine β-hydroxylase, 5 = catechol-O-methyltransferase, 6 = dihydropteridine reductase and 7 = tetrahydrobiopterin biosynthetic pathway. GTP = guanosine triphosphate, BH4 = tetrahydrobiopterin, qBH2 = quinonoid dihydrobiopterin, B6 = pyridoxal-5'-phosphate, C = ascorbic acid, SAM = S-adenosylmethionine, SAH = S-adenosylhomocysteine, 3MT = 3-methoxytyrosine.

tyrosine was 326 (67–586) ng/ml (p = 0·02). In four of the six patients, administration of levodopa (or the presence of endogenously high values in L-ADDC deficiency) caused the CSF S-adenosylmethionine concentrations to fall below the normal range.

If a single outlier is excluded (patient 3, on levodopa treatment) a simple linear regression of CSF Se-adenosylmethionine upon 3-methoxytyrosine is evident (p = 0·02) (fig 1).

CSF methionine concentration showed no change with alteration of central levodopa concentration, mean change −2·5 μM (−13·5 − +8·5) (p = 0·58) and CSF 5-methyltetrahydrofolate concentrations were within the normal range for all samples.

Discussion

All the patients studied here had deficiencies of the central monoamine neurotransmitters, dopamine and serotonin. Children with dihydropteridine reductase deficiency (enzyme 6, fig 2) and pterin synthesis defects (pathway 7, fig 2) are unable to maintain sufficient concentrations of the cofactor tetrahydrobiop-
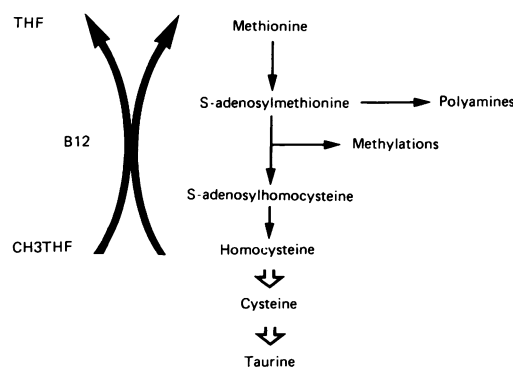

Figure 3 The formation and metabolic roles of S-adenosylmethionine. CH3THF = 5-methyltetrahydrofolate, SAM = S-adenosylmethionine, B12 = methylcobalamin, THF = tetrahydrofolate.

terin to ensure adequate hydroxylation of tyrosine and tryptophan for levodopa and 5-hydroxytryptophan formation and subsequent biogenic amine synthesis.[3] Treating these patients with levodopa and 5-hydroxytryptophan overcomes the metabolic block and corrects the amine deficiency. Patients with akinetic mutism have a general, unexplained dysfunction of central dopaminergic pathways and treatment with levodopa causes marked improvement in clinical signs.[11] Children with L-AADC deficiency (enzyme 3, fig 2) are unable to metabolise levodopa and 5-hydroxytryptophan and consequently are also amine deficient. In contrast to the other patients, brain concentrations of levodopa and 5-hydroxytryptophan are naturally raised.

Levodopa therapy caused a fall in CSF S-adenosylmethionine concentrations in all patients treated. Conversely, administration of pyridoxine to the patients with L-AADC deficiency (leading to a fall in CSF levodopa concentrations) produced a rise in CSF S-adenosylmethionine. This suggests that in these patients the endogenously formed levodopa was also causing a decrease in S-adenosylmethionine concentrations in the central nervous system.

The main pathway for the metabolism of exogenously administered levodopa involves methylation to 3-methoxytyrosine using S-adenosyl-methionine as the methyl-group donor.[5] This was confirmed in our patients where administration of levodopa (or the presence of endogenous levodopa in the patients with L-AADC deficiency) led to the formation of 3-methoxytyrosine. With one exclusion there was a linear relationship between CSF S-adenosylmethionine and 3-methoxytyrosine concentrations. In the one patient where this relationship did not hold, the initial S-adenosylmethionine concentration, before levodopa therapy, was very high. Administration of levodopa caused the largest fall in S-adenosylmethionine and this was associated with the largest rise in 3-methoxytyrosine concentration; suggesting that here too there was a relationship between S-adenosylmethionine and 3-methoxytyrosine concentrations. The finding of this linear relationship between S-adenosylmethionine and 3-methoxytyrosine demonstrates that when levodopa concentrations are raised the rate of its methylation is a major factor governing the S-adenosylmethionine concentration in the central nervous system.

We have previously demonstrated that profound deficiency of brain 5-methyltetrahydrofolate can cause a fall in CSF S-adenosylmethionine concentrations in humans.[12] Also others have shown in rodents that administration of levodopa leads to a greater fall in brain S-adenosylmethionine concentrations when the animals are folate deficient than when folate replete.[13] Patients with dihydropteridine reductase deficiency are known to develop a central nervous system folated deficiency which contributes to the neurological disease.[14] However, our patients are all receiving folinic acid replacement therapy. To check that folate

deficiency was not contributing to the reduced S-adenosylmethionine concentrations, 5-methyltetrahydrofolate was measured in the CSF. In all the patients, CSF 5-methyltetrahydrofolate concentrations were normal at the time of collection of CSF for S-adenosylmethionine and 3-methoxytyrosine estimation (table 2).

The decrease in CSF S-adenosylmethionine concentration was not accompanied by a decrease in methionine concentration. This presumably reflects the ability of the brain to maintain methionine concentrations by regeneration from homocysteine (fig 3). Similar findings have been observed in rodents,[15] in which the ability of the brain to buffer the increased demand for methyl groups caused by levodopa administration is via a folate dependent pathway.[13 16]

S-adenosylmethionine plays a key role in many metabolic pathways. These include methyltransfer reactions, aminopropylation reactions to form the polyamines and as a precursor for the transulphuration pathway[1] (fig 3). Evidence that decreased turnover of S-adenosylmethionine may be important in the pathogenesis of human neurological disease is suggested by the pharmacological effect of S-adenosylmethionine in depression[17] and our recently reported findings that it may be the cause of the demylination seen in inborn errors of folate metabolism.[12] The linear relationship between central S-adenosylmethionine and 3-methoxytyrosine concentrations indicates that the higher the dose of levodopa given, the greater the risk of depressing central S-adenosylmethionine concentrations, and that the dose of levodopa should be the lowest that gives the desired clinical effect.

The present results suggest that it is important to consider administration of S-adenosylmethionine, or other methyl-donors such as methionine and betaine, as an adjunct to levodopa therapy. Both betaine and methionine have been shown to be effective in in-born errors of cobalamin and folate metabolism where decreased concentrations of central nervous system S-adenosylmethionine are to be expected,[12 18] and, in animals, methionine supplementation can prevent the fall in brain S-adenosylmethionine concentrations induced by levodopa.[19]

1 Zappia V, Salvatore F, Porcelli M, Cacciapuoti G. Novel aspects in the biochemistry of adenosylmethionine and related sulphur compounds. In: Zappia V, Usdin E, Salvatore F, eds. *Biochemical and pharmacological roles of adenosylmethionine and the central nervous system.* Oxford: Pergamon Press, 1979:1–16.

2 Martindale. *The extra pharmacopoeia,* 28th ed. In: J E F Reynolds, ed. London: The Pharmaceutical Press, 1982:883–92.

3 Smith I. The hyperphenylalaninaemias. In: Lloyd JK, Scriver CR, eds. *Genetic and metabolic disease in pediatrics.* London: Butterworth, 1985:166–211.

4 Lovenberg W, Weissbach H, Udenfriend S. Aromatic L-amino acid decarboxylase. *J Biol Chem* 1962;237:89–93.

5 Sharpless NS, Muenter MD, Tyce GM, Owen CA. 3-methoxy-4-hydroxyphenylalanine (3-0-methylDOPA) in plasma during oral L-DOPA therapy of patients with Parkinson's disease. *Clin Chim Acta* 1972;37:359–69.

6 Wurtman RJ, Rose CM, Matthysse S, Stephenson J, Baldessarini R. L-Dihydroxyphenylalanine: effect on S-adenosylmethionine in brain. *Science* 1970;169:395–7.

7 Chalmers JP, Baldessarini RJ, Wurtman RJ. Effect of L-DOPA on norepinephrine metabolism in the brain. *Proc Natl Acad Sci (USA)* 1971;68:662–6.

8 Straementinoli G, Catto C, Algeri S. Decrease of noradrenaline 0-methylation in rat brain induced by L-DOPA. Reversal effect of S-adenosylmethionine. *J Pharm Pharmacol* 1980;32:430–1.

9 Surtees R, Hyland K. A new method for the measurement of S-adenosylmethionine in small volume samples of cerebrospinal fluid or brain using high performance liquid chromatography-electrochemistry. *Anal Biochem* 1989;181:331–5.

10 Joseph M, Davies P. Electrochemical activity of o-phthaldehyde-mercaptoethanol derivatives of amino-acids. *J Chromatogr* 1983;277:125–36.

11 Echiverri HC, Tatum WO, Merens TA, Coker SB. Akinetic mutism: pharmacological probe of the dopaminergic mesencephalofrontal activating system. *Pediatr Neurol* 1988;4:228–30.

12 Hyland K, Smith I, Bottiglieri T, Perry J, Wendel U, Clayton PT, Leonard JV. Demylination and decreased S-adenosylmethionine in 5,10-methylenetetrahydrofolate reductase deficiency. *Neurology* 1988;38:459–62.

13 Ordonez LA, Wurtman RJ. Folic acid deficiency in methyl group metabolism in brain: effects of L-Dopa. Reversal effect of S-adenosylmethionine. *Arch Biochem Biophys* 1974;160:372–6.

14 Smith I, Hyland K, Kendall B, Leeming R. Clinical role of pteridine therapy in tetrahydrobiopterin deficiency. *J Inher Metab Dis* 1985;8(suppl 1):39–45.

15 Ordonez LA, Wurtman RJ. Methylation of exogenous 3,4-dihydroxyphenylalanine (L-DOPA): effects on methyl group metabolism. *Biochem Pharmacol* 1973;22:134–7.

16 Ordonez LA, Wurtman RJ. Enzymes catalysing the de novo synthesis of methyl groups in the brain and other tissues of the rat. *J Neurochem* 1973;21:1447–55.

17 Carney MWP. Neuropharmacology of S-adenosyl methionine. *Clin Neuropharmacol* 1986;9:235–45.

18 Bartholomew DW, Batshaw ML, Allen RH, Roe CR, Rosenblatt D, Valle DL, Francomano CA. Therapeutic approaches to cobalamin-C methylmalonic acidemia and homocystinuria. *J Pediatr* 1988;112:32–9.

19 Taufek H, Bone A. Influence of exogenous L-3,4-dihydroxyphenylalanine (L-DOPA) on the methionine and S-adenosylmethionine concentrations in the brain and other tissues. *Biochem Soc Trans* 1980;8:62–3.