Communications to the Editor

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THE FORMATION OF 3-HYDROXY-4-METHOXYPHENYLALANINE AND 3-HYDROXY-4-METHOXYPHENETHYLAMINE IN PLASMA DURING L-DOPA THERAPY IN PATIENTS WITH PARKINSON'S DISEASE

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After oral administration of L-3,4-dihydroxyphenylalanine to patients with Parkinson's disease, 3-hydroxy-4-methoxyphenylalanine and 3-hydroxy-4-methoxyphenethylamine were identified as plasma metabolites, by high performance liquid chromatograph with fluorimetric detection.

KEYWORDS —— 3,4-dihydroxyphenylalanine; 3-hydroxy-4-methoxyphenylalanine; 3-hydroxy-4-methoxyphenethylamine; Parkinson's disease; fluorescence high performance liquid chromatography

Catecholamines (CA) are important components of the central nervous system. A number of disease are characterized by abnormal levels of CA; for example, patients with Parkinson's disease have lower levels of dopamine (DA) than normal.[1] L-3,4-Dihydroxyphenylalanine (L-DOPA) a catechol α-amino acid, is widely used in the treatment of Parkinson's disease.[2] When L-DOPA is given orally to patients, the most prominent metabolite is 3-methoxy-4-hydroxyphenylalanine (3-O-methyl-DOPA).[3] *In vitro*, experiments revealed that 3-O- and 4-O-methylated catechol derivatives may also be formed by enzymatic methylation of catechol-O-methyltransferase (COMT).[4] *In vivo*, however, O-methylation occurs almost exclusively on the 3-O-methylated catechol derivatives.[5] Furthermore, L-DOPA itself does not convert to 3-hydroxy-4-methoxyphenylalanine (4-O-methyl-DOPA) in mammals.[6] Recently, we reported the formation of 4-O-methyl-DOPA from DOPA *in vitro* using rat liver homogenate.[7] However, the formation of 4-O-methyl-DOPA *in vivo* was not then detected.[8] The present communication describes the formation of 4-O-methyl-DOPA, including 3-hydroxy-4-methoxyphenethylamine (4-O-methyl-DA), following oral L-DOPA therapy in Parkinson patients.

Two patients with Parkinson's disease, 71 and 72 years old, participated in this study. L-DOPA was administrated orally at either 0.4 or 1.4 g per day. The L-DOPA is rapidly absorbed, and a maximum plasma concentration of 3-O-methyl-DOPA as a major metabolite of L-DOPA was attained 3 h after drug administration;[3] therefore, in the present study, venous blood was collected with a syringe at this

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time. The sample was placed in heparinized tubes and centrifuged at 1000 × g for 5 min at 4°C. A 2 ml sample of plasma was deproteinized by the addition of 0.5 ml of 1.0M trichloroacetic acid. After centrifuging down the precipitate, 50 µl of the supernatant was subjected to fluorescence high performance liquid chromatography (HPLC). Typical chromatograms of normal and patient plasma are shown in Fig. 1. These chromatographic analyses indicate that the concentration

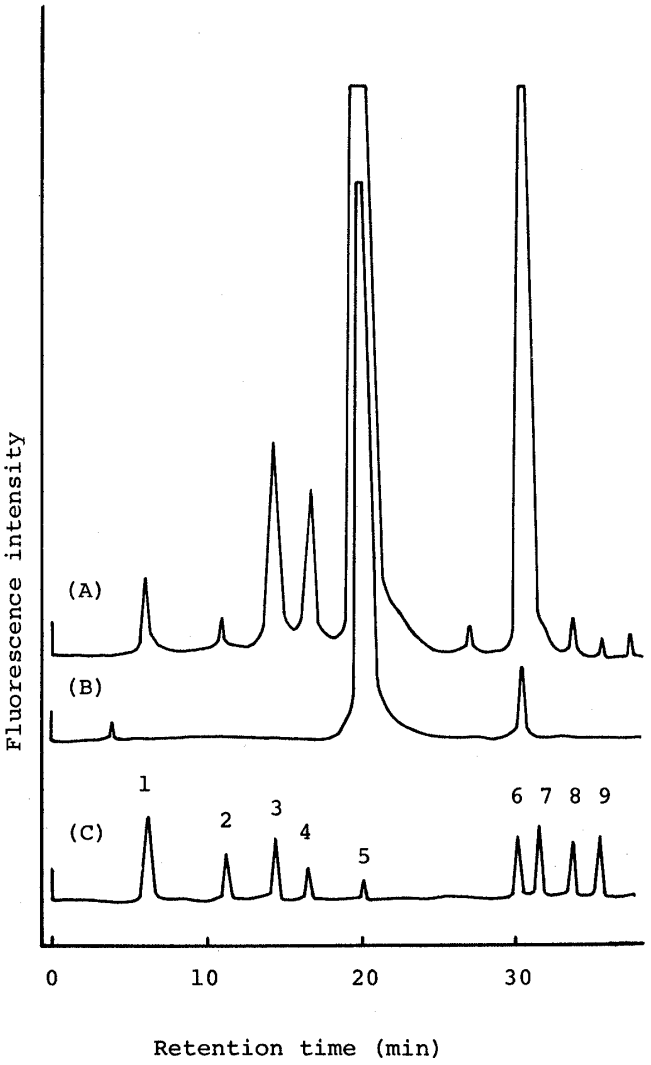

Fig. 1. Chromatograms of HPLC of Human Plasma

(A) Injection sample: Plasma sample obtained from a Parkinson patient 3 h after administration of L-DOPA.

(B) Injection sample: Normal plasma sample obtained from healthy person.

(C) Injection sample: Standard solution containing 100 ng/ml each of the compounds studied.

A 50 µl portion was introduced to HPLC under the following conditions: column, Yanapak ODS (250 mm × 4.0 mm I.D.); mobile phase, 0.05 M phosphate buffer (pH 3.1) was used for the first 6 min, then a linear gradient of increasing methanol concentration to 15% methanol phosphate buffer was applied over 6-36 min; flow rate, 0.54 ml/min; fluorescence detection at 282 nm/322 nm.

Peaks: 1 = noradrenaline; 2 = adrenaline; 3 = DOPA; 4 = DA; 5 = p-tyrosine; 6 = 3-O-methyl-DOPA; 7 = 3-O-methyl-DA; 8 = 4-O-methyl-DOPA; 9 = 4-O-methyl-DA.

of DOPA, DA, 4-O-methyl-DOPA and 4-O-methyl-DA in the plasma was significantly increased by oral administration of L-DOPA. Under these chromatographic conditions, the retention time of noradrenaline, adrenaline, 3-O-methyl-DOPA and 3-O-methyl-DA, clearly different from those of DOPA, DA, 4-O-methyl-DOPA and 4-O-methyl-DA.

The plasma concentrations of DOPA, DA, 4-O-methyl-DOPA and 4-O-methyl-DA after oral administration of L-DOPA in two patients are shown in Table I. A part of

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Table I. The Plasma Concentrations of DOPA, DA, 4-O-Methyl-DOPA and 4-O-Methyl-DA 3 h after Oral Administration of L-DOPA in Parkinson Patients

Subject	Dose of L-DOPA	Plasma concentration (ng/ml)			
	g/day	DOPA	DA	4-O-Methyl-DOPA	4-O-Methyl-DA
1	0.4	42.1	37.2	6.3	1.9
2	1.4	378.3	298.9	15.2	5.4

the orally administered L-DOPA is methylated to 4-O-methyl-DOPA and 4-O-methyl-DA (Table I). Previously,[9] Friedhoff and van Winckle reported that the 4-O-methyl reaction of CA is implicated in some neuropsychiatric disorders. Thus, 4-O-methyl-DA appears to be the endogeneous "toxin" in Parkinsonism.[10] Consequently, the determination of plasma levels of 4-O-methyl-DOPA and 4-O-methyl-DA is important following oral L-DOPA therapy.

The present study suggests that 4-O-methyl-DOPA and 4-O-methyl-DA are also formed from L-DOPA in vivo. More detailed investigations of the metabolism in patients with Parkinson's disease and therapeutic monitoring are now in progress in our laboratory.

REFERENCES

1) C. Curzon, in Cummings (Ed.),"Biochemical Aspects of Nervous Disease," Plenum Press, New York, 1972, p. 151.
2) a) G.C. Cotzias, P.S. Papavasiliou, and R. Gellene, N. Engl. J. Med., 280, 337 (1969); b) M.D. Yahr, R.C. Duvoisin, M.J. Schear, R.E. Banett, and M.M. Hoehn, Arch. Neurol., 21, 343 (1969); c) F. McDowell, J.E. Lee, T. Swift, R.D. Sweet, J.S. Ogsbury, and J.T. Kesselr, Ann. Intern. Med., 72, 29 (1970); d) J. Marka, "The Treatment of Parkinsonism with L-DOPA," Elsevier, New York, 1974.
3) N.S. Sharpless, M.D. Muenter, G.M. Tyce, and C.A. Owen, Jr., Clin. Chim. Acta, 37, 359 (1972).
4) a) S. Senoh, J.W. Daly, J. Axelrod, and B. Witkop, J. Am. Chem. Soc., 81, 6240 (1959); b) J.W. Daly, J. Axelrod, and B. Witkop, J. Biol. Chem., 235, 1155 (1960); c) C.R. Creveling, N. Dalgard, H. Shimizu, and J.W. Daly, Mol. Pharmacol., 6, 691 (1970); d) Y.-Q. Qu, K. Imai, Z. Tamura, Y. Hashimoto, and H. Miyazaki, Life Sci., 32, 1811 (1983).
5) J. Axelrod, S. Senoh, and B. Witkop, J. Biol. Chem., 233, 697 (1958).
6) P. Mathieu, J.C. Charvet, and J. Greffe, Biochem. Pharmacol., 24, 43 (1975).
7) T. Ishimitsu, and S. Hirose, Chem. Pharm. Bull., 28, 2272 (1980).
8) T. Ishimitsu, and S. Hirose, Chem. Pharm. Bull., 29, 3400 (1981).
9) a) A.J. Friedhoff, and E. van Winckle, Nature, 194, 897 (1962); b) A.J. Friedhoff, and E. van Winckle, J. Nerv. Ment. Dis., 135, 550 (1962).
10) B.C. Barras, D.B. Coult, and R.M. Pinder, J. Pharm. Pharmacol., 24, 499 (1972).

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