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Department of Pharmacology,
Faculty of Pharmaceutical Sciences,
Osaka University,
Toneyama,
Toyonaka,
Osaka-fu,
Japan

HEITAROH IWATA
ITARU YAMAMOTO
EIICHI GOHDA
KYOJI MORITA
KIYOKO NISHINO

REFERENCES

1. H. IWATA, I. YAMAMOTO and K. MURAKI, *Biochem. Pharmac.* **18,** 955 (1969).
2. H. KLENOW and R. EMBERLAND, *Archs Biochem. Biophys.* **58,** 276 (1955).
3. P. PLESNER and H. M. KALCKAR, in *Methods of Biochemical Analysis* (Ed. D. GLICK), Vol. III, p. 97, Interscience, New York (1956).
4. E. DELLA CORTE and F. STIRPE, *J. Biochem.* **108,** 349 (1968).
5. I. FRIDOVICH, *J. biol. Chem.* **140,** 2491 (1965).
6. W. N. KELLER, F. M. ROSENBLOOM, J. F. HENDERSON and J. E. SEEGMILLER, *Proc. natn. Acad. Sci. U.S.A.* **57,** 1735 (1967).
7. Y. F. SHEALY, C. A. KRAUTH and J. A. MONTGOMERY, *J. org. Chem.* **27,** 2150 (1962).
8. H. BRANDENBERGER, *Biochim. biophys. Acta* **15,** 108 (1954).
9. R. TRUSCOE and V. WILLIAMS, *Biochem. Pharmac.* **17,** 165 (1968).
10. K. HANO, A. AKASHI, I. YAMAMOTO, S. NARUMI and H. IWATA, *Gann* **59,** 207 (1968).
11. Y. F. SHEALY, R. F. STRUCK, L. B. HOLUM and J. A. MONTGOMERY, *J. org. Chem.* **26,** 2396 (1961).
12. W. N. KELLEY, F. M. ROSENBLOOM, J. MILLER and J. E. SEEGMILLER, *New Eng. J. Med.* **278,** 287 (1968).
13. R. J. MCCOLLISTER, W. R. GILBERT, D. M. ASHTON and J. B. WYNGAARDEN, *J. biol. Chem.* **239,** 1560 (1964).

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Effects of peripheral aromatic L-amino acids decarboxylase inhibitor on L-[2-^{14}C]-3,4-dihydroxyphenylalanine metabolism in man

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ONE CURRENT approach to the treatment of Parkinson's disease is focused on the possible combination of L-3,4-dihydroxyphenylalanine (L-dopa) with an inhibitor of peripheral aromatic L-amino acids decarboxylase. This approach attempts to reduce[1–3] the therapeutic dose of L-dopa by inhibiting its extracerebral decarboxylation, thus minimizing certain dopa-induced side effects. It has been shown previously[1–3] that certain aromatic hydrazine-type compounds may interfere in the metabolism of exogenous L-dopa by inhibiting peripheral dopa-decarboxylase (DC). Examples of such compounds are: RP-4-4602 [*N*-(DL-seryl)-*N'*-(2,3,4-trihydroxybenzyl)hydrazine], MK-485[DL-α-hydrazino-α-methyl-β-(3,4-dihydroxyphenyl) propionic acid] and NSD-1015 (*m*-hydroxybenzylhydrazine). Animal experiments have demonstrated that although MK-485 is a less potent inhibitor than an equimolar dose of RO-4-4602 or NSD-1015, it has the advantage of not penetrating into the brain,[4] hence cerebral DC remains unaffected.

The present investigation studied the effect of pretreatments with MK-486, the L-isomer of MK-485, on blood plasma and urinary excretion patterns of L-[2-^{14}C]-dopa and metabolites in three Parkinsonian patients free of renal, hepatic or cardiovascular disease. All medication was discontinued 1 week

prior to the beginning of the study. After an overnight fast, 50 μc L[2-^{14}C]-dopa and 100 mg of authentic L-dopa were administered to each patient. Blood and urine samples were collected serially during an 8-h period. Plasma samples were assayed for dopa and its metabolites, dopamine (DA) and homovanillic acid (HVA). Urine specimens were analyzed for total (free and conjugated) DA[5], free dihydroxyphenylacetic acid (DOPAC) and HVA. The resulting metabolic profile was compared with that obtained with an identical dose of L-dopa a week later, but 1 hr after pretreatment with MK-486, 100 mg in a single dose. Pretreatment with MK-486, 100 mg three times per day for 7 days, preceded the third dose of L-[2-^{14}C]-dopa and authentic L-dopa.

The fractionation technique for the isolation of dopa and its urinary metabolites, DA, DOPAC[6] and HVA, is reported in detail elsewhere.[7] The quantitative determinations of dopa and DA followed a modification[8,9] of the trihydroxyindole reaction.[10] DOPAC and HVA were assayed as previously described by the procedures of Mellinger and Hvidberg[11] and Sato[12] respectively.

The effects of MK-486 on the mean plasma concentrations of dopa, DA and HVA as a function of time are displayed in Fig. 1. After the administration of L-dopa alone (referred to as baseline), plasma dopa concentrations were barely detectable during the entire 8-hr period. Pretreatment with MK-486 in a single 100-mg dose increased the mean peak plasma dopa concentration to 0·7 μg/ml, while pretreatment with MK-486 for 1 week resulted in a further increase to 1·2 μg/ml. Conversely, the baseline plasma DA concentration, which peaked at 0·3 μg/ml during a 2-hr period, was diminished by MK-486 pretreatment to a level below the sensitivity of the analytical procedure (2·5 ng/ml of reaction mixture). Pretreatment with a single dose of MK-486 reduced the plasma HVA concentration to approximately 80 per cent of the baseline value. Pretreatment with the inhibitor for 1 week diminished the measurable plasma HVA concentration further.

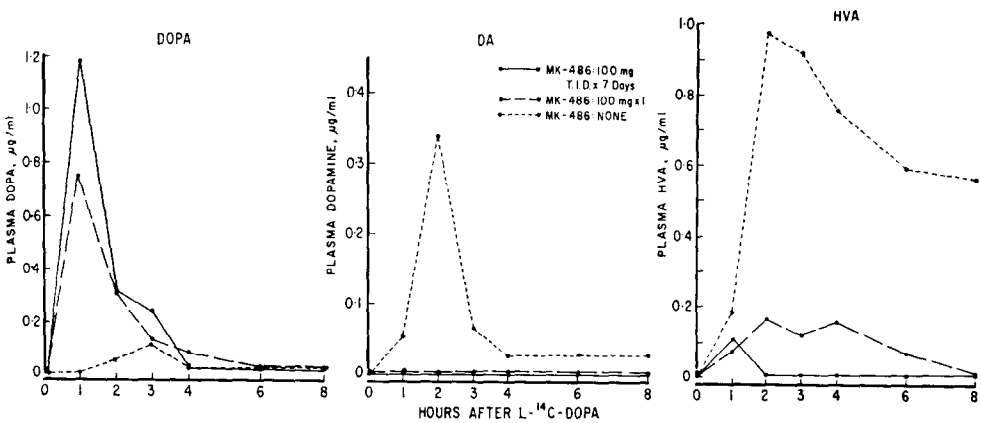

FIG. 1. Effect of MK-486 pretreatment on plasma dopa (left panel), dopamine (middle panel) and homovanillic acid (right panel) after administration of L-[2-^{14}C]-dopa. Plasma dopa, dopamine and homovanillic acid, expressed as micrograms per milliliter, are plotted against time.

Urinary excretion profiles of DA, DOPAC and HVA as modified by MK-486 are shown in Fig. 2. Urinary DA levels were markedly reduced by MK-486 pretreatment. For example, during the first 2 hr, baseline urinary excretion of DA (2·2 mg) was decreased to 0·6 and 0·2 mg by single and multiple dose pretreatment with MK-486, respectively. The baseline urinary excretion pattern of DOPAC was similarly decreased by MK-486 pretreatment. Peak urinary HVA levels were decreased by approximately 70 per cent with MK-486 pretreatment.

The above data were not corrected for the endogenous amounts of DA, DOPAC and HVA. However, radioactivity derived from L-[2-^{14}C]-dopa, as measured in the catecholamines, DOPAC and acid fractions (Table 1), parallels the quantitative determinations of these compounds (Figs. 1 and 2). Data in Table 1 are expressed as a ratio of radioactivity in plasma, urine and certain fractions of urine obtained after pretreatment with MK-486 relative to corresponding radioactivity measured without MK-486 pretreatment. In plasma, these ratios of total radioactivity increase as a function of time to values greater than two at 6 and 8 hr. Conversely, this ratio in urine is less than one at all times studied. This suggests that MK-486 pretreatment, either in single or multiple doses, markedly enhances the appearance of radioactivity in plasma but delays the excretion of radioactivity in urine.

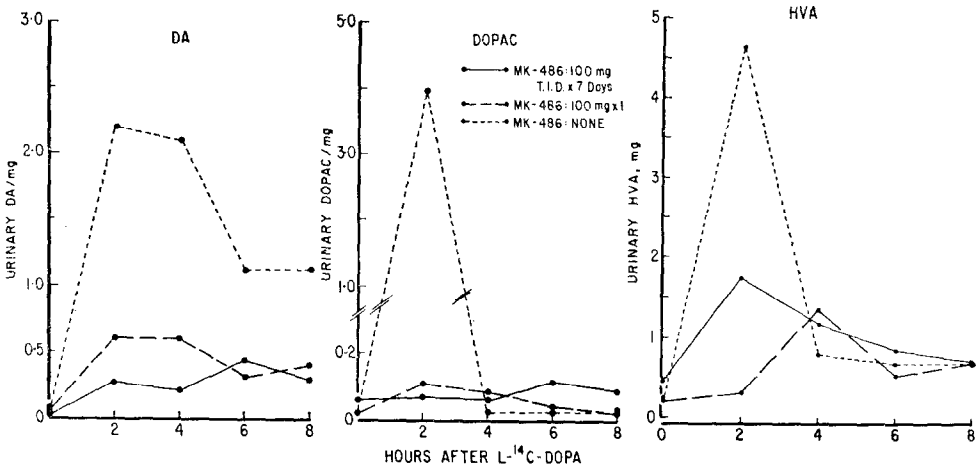

FIG. 2. Effect of MK-486 pretreatment on the excretion of urinary dopamine (left panel), dihydroxyphenylacetic acid (middle panel) and homovanillic acid (right panel) after administration of L-[2-^{14}C]-dopa. Urinary dopamine, dihydroxyphenylacetic acid and homovanillic acid, expressed as milligrams, are plotted against time.

TABLE 1. EFFECT OF MK-486 PRETREATMENT ON RADIOACTIVITY DERIVED FROM L-[2-^{14}C]-DOPA PRESENT IN PLASMA, URINE AND CERTAIN FRACTIONS OF URINE*

		MK-486							
		Single dose (100 mg)				100 mg three times daily for 1 wk			
Analytical assay fraction	Specimen	Hours after L-2-^{14}C-dopa				Hours after L-2-^{14}C-dopa			
		2	4	6	8	2	4	6	8
Total ^{14}C	Plasma	0·97	1·48	2·20	2·16	0·69	1·62	2·34	2·95
Total ^{14}C	Urine	0·31	0·64	0·71	0·64	0·29	0·39	0·90	0·78
^{14}C-dopa	Urine	0·44	0·91	0·77	0·72	0·59	0·57	1·26	0·92
^{14}C-catecholamines	Urine	0·19	0·30	0·29	0·39	0·13	0·12	0·47	0·39
^{14}C-acids	Urine	0·29	0·82	0·75	0·67	0·25	0·50	1·00	0·86
^{14}C-dopac	Urine	0·23	0·35	0·40	0·46	0·20	0·32	0·39	0·45

* Data are expressed as ratio of radioactivity modified by MK-486 pretreatment relative to corresponding radioactivity measured without MK-486 pretreatment.

Further, a corresponding delay in urinary excretion of radioactive dopa, catecholamines, DOPAC and acid metabolite fractions is noted.

Treatment with MK-486 prior to L-[2-^{14}C]-dopa administration in man increased the plasma concentration of dopa and concomitantly decreased both plasma DA and the major acid metabolite, HVA. These findings suggest that MK-486 is an effective inhibitor of dopa decarboxylase in man. If, as suggested,[13,14] MK-486 preferentially inhibits enzymatic decarboxylation of aromatic L-amino acids, then our data may suggest that L-dopa decarboxylation in our subjects was predominantly an enzymatic rather than a nonenzymatic[15] process.

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F. S. MESSIHA
T. H. HSU
J. R. BIANCHINE

REFERENCES

1. W. BIRKMAYER, *Klin. Wschr.* **81,** 677 (1969).
2. G. C. COTZIAS, P. S. PAPAVASILIOU and R. GELLENE, *New Engl. J. Med.* **280,** 337 (1969).
3. R. TISSOT, J. M. GILLARD, M. GUGGISBERG, G. GAUTHIER and J. E. AJURIAUERRA, *Presse med.* **77,** 619 (1969).
4. G. BARTHOLINI and A. PLETSCHER, *J. Pharm. Pharmac.* **21,** 323 (1969).
5. F. S. MESSIHA, D. AGALLIANOS and C. CLOWER, *Nature, Lond.* **225,** 868 (1970).
6. F. S. MESSIHA, J. R. BIANCHINE, T. H. HSU, B. ENCOMIENDA and V. BULANHAGUI, *Fedn Proc.* **30,** 224 (1971).
7. F. S. MESSIHA, T. H. HSU and J. R. BIANCHINE, *J. clin. Invest.* **51,** 452 (1972).
8. R. LAVERTY and K. M. TAYLOR, *Analyt. Biochem.* **22,** 269 (1968).
9. F. S. MESSIHA, R. W. VON KORFF, *Fedn Proc.* **28,** 543 (1969).
10. A CARLSSON and B. WALDECK, *Acta physiol. Scand.* **44,** 293 (1958).
11. T. J. MELLINGER and E. F. HVIDBERG, *Am. J. clin. Path.* **51,** 559 (1969).
12. T. L. SATO, *J. Lab. clin. Med.* **66,** 517 (1965).
13. C. C. PORTER, *Fedn Proc.* **30,** 871 (1971).
14. A. PLETSCHER and G. BARTHOLINI, *Clin. Pharmac. Ther.* **12,** 344 (1971).
15. W. H. VOGEL, *Naturwissenschaften* **56,** 462 (1969).

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Methemoglobin—Induced by carcinogenic aminoazo dyes in rats

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WHEN a single dose ($3{\cdot}23 \times 10^{-4}$ moles/kg of body wt) of 4-aminoazobenzene (AB) was administered intraperitoneally to rats of the Sprague–Dawley strain, the animals eventually developed typical symptoms of cyanosis, as revealed by color changes in the eyeball, mucous membrane and tip of the toe, and occasionally by the presence of exertional dyspnea. Less severe symptoms were also observed in rats given the hepatocarcinogens, *N*-monomethyl-4-aminoazobenzene (MAB) or *N,N*-dimethyl-4-aminoazobenzene (DAB). The cyanotic symptoms suggest that certain hematological changes in the animals were induced by these dyes. Many arylamino and nitro compounds have been shown to be highly active in forming methemoglobin (MHb).[1,2] This prompted us to study the MHb-forming ability of aminoazo dyes.

The concentration of MHb in the blood of rats treated with AB, MAB or DAB was determined by the method of Evelyn and Malloy.[3] The results are given in Fig. 1. Aniline, *N*-monomethylaniline and *N,N*-dimethylaniline were used as controls for the purpose of comparison, since they have identical functional groups with AB, MAB and DAB respectively. With the same molar concentration ($3{\cdot}23 \times 10^{-4}$ moles/kg), the maximum levels of MHb induced by AB, MAB, DAB, aniline, *N*-mono-