

Peripheral Aromatic L-Amino Acids Decarboxylase Inhibitor in Parkinsonism

I. EFFECT ON O-METHYLATED METABOLITES OF L-DOPA-2-¹⁴C

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ABSTRACT The effects of MK-486, an inhibitor of peripheral aromatic L-amino acids decarboxylase, on the urinary metabolites derived from orally administered L-Dopa-2-¹⁴C were studied in three Parkinsonian patients. Treatment with MK-486 before L-Dopa-2-¹⁴C markedly reduced radioactivity found in catecholamines fraction by 70–80% during 48 hr, but increased 3-O-methyl-dopa fraction by threefold, as compared with a nonpretreated base line value. Pretreatment with MK-486 for a period of 1 wk resulted in less inhibition of O-methylated amine and acid metabolite fractions than that measured after a single dose of the inhibitor.

INTRODUCTION

Some adverse effects arising from Levodopa therapy in Parkinsonism might be minimized by reducing Levodopa dose requirements with an inhibitor of peripheral aromatic L-amino acids decarboxylase (1–3). Since DOPA decarboxylase (E.C.4.1.1.26) activity (DC),¹ found in various species, is greater in peripheral organs than

in the brain (4–6), most orally administered Levodopa is decarboxylated peripherally. The potential efficacy of aromatic L-amino acids decarboxylase inhibitor is dependent upon preferential inhibition of extracerebral decarboxylation of Levodopa and relative impenetrability of the inhibitor into the brain. The importance of Levodopa in the treatment of Parkinsonism is well known (7). Therefore, we studied the L-isomer of an inhibitor of aromatic L-amino acids decarboxylase, MK-486, L- α -hydrazino- α -methyl- β -(3,4-dihydroxyphenyl) propionic acid. This investigation reports some metabolic effects of MK-486 on the excretion profiles of urinary metabolites derived from L-Dopa-2-¹⁴C by three Parkinsonian patients.

METHODS

Clinical. Three male Parkinsonian patients, (aged 58, 65, 68 yr), whose conditions were uncomplicated by renal, hepatic, or cardiovascular disease were admitted to the Clinical Pharmacology Research Unit for this study. All medications were discontinued for 7 days before the study. After an overnight fast, each patient then received by mouth a single capsule containing 50 μ Ci (100 mg) L-Dopa-2-¹⁴C which was purchased from Amersham/Searle Corp. (Arlington Heights, Ill.), added to nonradioactive Levodopa and recrystallized from water until constant specific activity was reached (0.5 μ Ci/mg). Radiochemical and chemical purity was further established by melting points, UV absorption, and thin-layer chromatography. Urine samples were collected every 2 hr during the first 8 hr and re-

Received for publication 13 August 1971 and in revised form 3 November 1971.

¹Abbreviations used in this paper: CA, catecholamines; DC, decarboxylase activity; DOPAC, dihydroxyphenyl-acetic acid; HVA, homovanillic acid; MK-486, L- α -hydrazino- α -methyl- β -(3,4-dihydroxyphenyl) propionic acid; 3-O-MD, 3-O-methyl-dopa; VMA, vanillylmandelic acid.

mainder of the first 24 hr as well as the following 24 hr period and were analyzed separately. 1 wk later similar urine collections were made after a single dose of 100 mg MK-486 administered 1 hr before the administration of the same dose of L-Dopa-2-¹⁴C. Pretreatment with the inhibitor in a dosage of 100 mg t.i.d. for 7 days preceded the third administration of the equal dose of ¹⁴C-labeled and authentic Levodopa and urine collections and tests were performed in the same manner.

Analytical techniques. The fractionation technique employed for the isolation of certain major metabolites of Levodopa is similar to that of Bartholini and Pletscher (8) originally designed for tissue assay. However, urinary fractionation required the following modification: portions of acid-hydrolyzed urine samples were chromatographed at pH 6.5 on a weak cation exchange resin, Amberlite CG-50, in Na⁺ cycle and washed successively with 0.1 M, pH 6.5 phosphate buffer followed by water. The monoamines were then eluted with 2.0 N acetic acid; and rechromatographed at pH 8.5 on alumina to separate the *O*-methylated amines, in effluents, from the absorbed catecholamines, which were subsequently eluted with 1.0 N acetic acid. A portion of each fraction was measured for radioactivity.

For the separation of *O*-methylated acid metabolites of Levodopa, further urine samples were subjected to chromatography at pH 4.5 on Dowex 1 × 4, in acetate cycle, where the acid metabolites were eluted with 2.0 M, NaCl solution. Portions of the latter were rechromatographed on alumina at pH 8.5 to absorb the dihydroxyphenyl acid metabolites, mainly dihydroxyphenylacetic acid, (DOPAC). After washings with water and by 0.1 N acetic acid, DOPAC was eluted with 1.0 N perchloric acid. Alumina effluents, containing mainly *O*-methylated acid metabolites, were acidified and saturated with NaCl before a double extraction with ethylacetate, which were combined. Portions of the latter were subjected to extraction with 3 N ammonium hydroxide which served for the quantitative determinations of homovanillic acid (HVA) as described by Sato (9). Vanillyl-

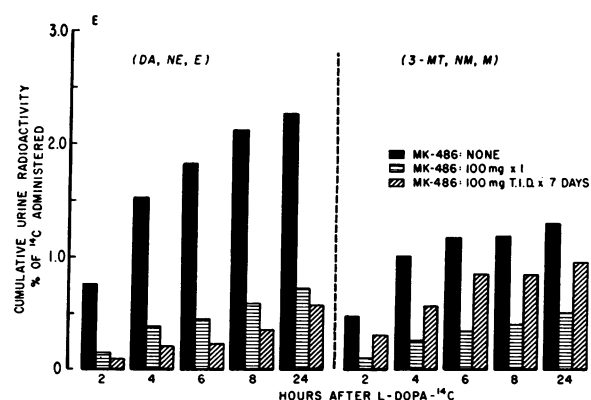


FIGURE 1 Effect of MK-486 pretreatment on the urinary excretion of catecholamines; i.e., dopamine (DA), norepinephrine (NE), and epinephrine (E) (left panel) and their 3-*O*-methylated metabolites; i.e., 3-methoxytyramine (3-MT), normetanephrine (NM), and metanephrine (M) (right panel), after administration of L-Dopa-2-¹⁴C. Total cumulative radioactivity present in urinary CA fraction (left panel) and their *O*-methylated metabolites (right panel) expressed as per cent of total radioactivity administered as a function of time.

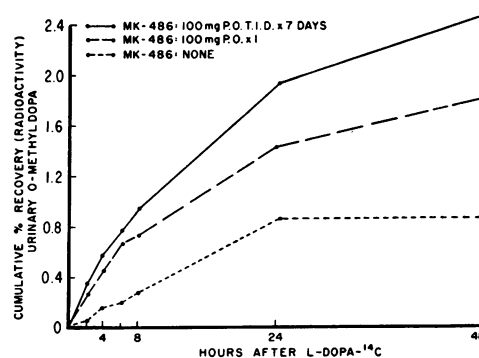


FIGURE 2 Effect of MK-486 pretreatment on cumulative urinary excretion of *O*-methylated dopamine (*O*-MD) after oral administration of L-Dopa-2-¹⁴C. Total cumulative radioactivity present in urinary *O*-methylated dopamine fraction, expressed as per cent of total radioactivity administered, as a function of time.

mandelic acid (VMA) determinations were performed as outlined by Pisano, Crout, and Abraham (10). Recovery of authentic internal standards utilizing this procedure ranged from 60 to 65% for HVA and VMA, 70–75% for the catecholamines and their respective *O*-methylated amine metabolites (3-methoxytyramine, normetanephrine, and metanephrine). The interference of MK-486 in the isolation and in analytical procedures described above was insignificant.

Portions of alumina eluents and effluents were also measured for radioactivity. Fractionation of 3-*O*-methylated dopamine (3-*O*-MD) followed the procedure of Wurtman, Chou, and Rose (11). However, 2.0 M, NaCl solution was utilized for elution of 3-*O*-MD from the cation exchange resin Dowex 50W-X4.

RESULTS

After the administration of L-Dopa-2-¹⁴C alone, base line radioactivity was measured in those fractions containing catecholamines (CA), their *O*-methylated monoamines and major *O*-methylated acid metabolites. 90% of the radioactivity administered was recovered in the urine within 48 hr. Acid and neutral metabolite fraction account for approximately 80% of this radioactivity. Pretreatment with MK-486 either in a single 100 mg dose or in 100 mg doses administered three times per day for 7 days decreased this recovery of radioactivity from urine to 63 and 65%, respectively and decreased acid and neutral fractions of urine to 56 and 63%, respectively.

Fig. 1 shows the effect of pretreatments with MK-486 on the cumulative excretion of urinary CA and their *O*-methylated metabolites, expressed in per cent of radioactivity of dose administered. The measured radioactivity in the CA fraction was markedly reduced by both single and multiple dose pretreatment with the inhibitor. After a single pretreatment dose of MK-486 a similar trend was observed in the *O*-methylated CA

metabolites. However, less inhibitory effect in the *O*-methylated CA fraction was noted after prolonged pretreatment with MK-486.

Conversely, radioactivity measured in the fraction containing mainly 3-*O*-MD, (Fig. 2), was increased by the pretreatment of a single dose of MK-486. This was further increased after prolonged pretreatment. Cumulative excretion of urinary 3-*O*-MD accounted for 2.5% of administered dose during 48 hr.

Fig. 3 illustrates the effect of MK-486 on the cumulative excretion of the CA and their *O*-methylated acid metabolite fraction derived from L-Dopa-2-¹⁴C. Data are expressed as per cent inhibition from nonpretreatment base line values. The CA fraction was equally reduced by each form of pretreatment with the inhibitor. This decrease approximated 70–80% of base line and remained relatively constant throughout a 48 hr period. Further, Fig. 3 indicates that the per cent inhibition of radioactivity measured in the *O*-methylated acid fraction (mainly HVA) was very marked during the first 8 hr period. This decrease progressively declined with time and contrasts to the constant inhibition observed in CA fraction during 48 hr period.

Quantitative determination of HVA indicated a similar decrease in per cent inhibition after pretreatment of the inhibitor. However, urinary VMA concentrations, which constituted a small fraction of the Levodopa dose administered, were not markedly altered by MK-486 pretreatment.

DISCUSSION

It has been suggested (12) that inhibitors of peripheral aromatic L-amino acids decarboxylase enhance a dopa-induced rise in cerebral CA while minimizing formation of extracerebral DOPA metabolites which might contribute to undesired side effects. On the other hand, treatment by Levodopa coupled with a peripheral DC inhibitor might also facilitate the formation of extracerebral DOPA metabolite(s) such as 3-*O*-MD which could penetrate into the brain (13). In the present study, a modest increase was noted in *O*-methylated CA and 3-*O*-MD fraction after pretreatment with the inhibitor. This finding is of particular interest since 3-*O*-MD itself is now in clinical trial (14).

Prolonged pretreatment with the inhibitor for 1 wk was designed in this study to saturate the peripheral pool of aromatic L-amino acids decarboxylase before the administration of the substrate, Levodopa. Thus, providing a comparison of initial vs. chronic effects of MK-486. Unexpectedly, there was no apparent difference between the MK-486 pretreatment schedules obtained regarding the marked inhibition found in CA fraction. This decrease remained relatively constant through a subsequent 48 hr period. MK-486 inhibitory

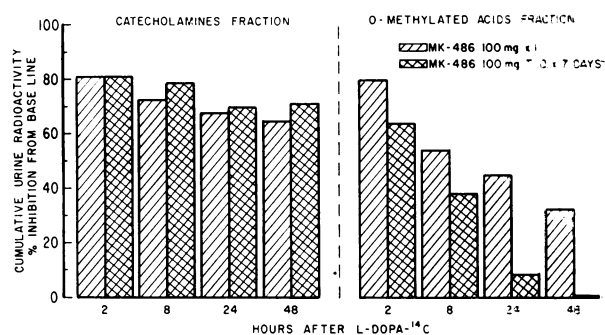


FIGURE 3 Effect of MK-486 pretreatment on the urinary excretion of catecholamines (left panel) and their *O*-methylated acid metabolites (right panel), after administration of L-Dopa-2-¹⁴C. Data represents per cent inhibition by MK-486 relative to nonpretreated base line excretion, plotted against time.

effect on the *O*-methylated acid fraction, was of short duration and declined rapidly with time. A similar trend was observed in the quantitative determination of HVA, while the excretion profile of VMA was not markedly changed by MK-486 treatment. The increase in the *O*-methylated acid metabolites after prolonged pretreatment with MK-486 might suggest that the metabolic conversion of DOPA to its major *O*-methylated acid metabolite preceded by transamination of the formed 3-*O*-MD to the corresponding lactic acid, through pyruvic acid, to form HVA as has been recently suggested by Bartholini, Kuruma, and Pletscher (15). However, one cannot exclude the possibility that prolonged pretreatment with the inhibitor might induce enzymes involved in the oxidative deamination of L-DOPA-methylated metabolite(s).

ACKNOWLEDGMENTS

We thank Dr. John C. Krantz, Jr., for his encouragement and helpful discussion, and B. Encomienda and V. Bulanagui for excellent technical assistance.

We gratefully acknowledge encouragement and financial support from the following: Dr. M. Jaffe, Merck Sharp & Dohme, West Point, Pa.; American Parkinsons' Disease Association; Dr. D. Lovett Chapter, M. Colwill, H. Friedberg, C. Friedman, W. Gettinger, Dr. N. Herman, B. Kairys, A. Lapidis, M. Quartner, Tuttle Trust Fund, and M. Weiss Fund.

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