Biochemical Pharmacology, Vol. 24, pp. 1317–1320. Pergamon Press, 1975. Printed in Great Britain.

# CONJUGATION OF L-DOPA AND ITS METABOLITES AFTER ORAL AND INTRAVENOUS ADMINISTRATION TO PARKINSONIAN PATIENTS*

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(*Received* 28 *August* 1974; *accepted* 22 *November* 1974)

**Abstract**—Tracer quantities of $^3$H-L-dopa were given either orally or intravenously to patients with Parkinson's disease. The metabolism of $^3$H-L-dopa was estimated by measurement of $^3$H-L-dopa and some of its metabolites in urine samples collected during the first 24 hr. The decarboxylation of $^3$H-L-dopa to $^3$H-dopamine was approximately the same when $^3$H-L-dopa was given by the two routes. The subsequent metabolism of the newly formed $^3$H-dopamine was markedly dependent upon the route of administration. Conjugation was the major metabolic route of $^3$H-dopamine derived from $^3$H-L-dopa given orally, while *O*-methylation and deamination of $^3$H-dopamine to $^3$H-homovanillic acid predominated when $^3$H-L-dopa was given intravenously. In contrast to the metabolism of tracer quantities of oral $^3$H-L-dopa, large oral doses of L-dopa (3·0 g/day) resulted in a smaller proportion of conjugated dopamine and a greater proportion of homovanillic acid. The conjugation of $^3$H-dopamine after tracer quantities of intravenous $^3$H-L-dopa was only slightly decreased by the concomitant administration of 3·0 g/day of oral L-dopa. There is evidence that conjugation occurs primarily in the gastrointestinal–hepatic system after oral administration of L-dopa. When conjugation is either suppressed by large quantities of oral L-dopa or avoided by intravenous L-dopa, the amounts of free dopamine as well as homovanillic acid are increased. Thus, under these conditions, more free dopamine is available to produce the peripheral side effects of L-dopa therapy.

The administration of L-dopa to Parkinsonian patients has sometimes led to a variable and unpredictable response [1, 2]. This has been attributed, at least in part, to possible variations in: (1) gastric acidity and emptying time [3–5], (2) intestinal decarboxylation of dopa to dopamine [6] and (3) subsequent deamination and *O*-methylation of dopamine [7, 8]. Another major metabolic pathway for the metabolism of dopamine is conjugation with sulfuric acid [9–13], and we have preliminary observations that there is considerable variation between patients in the amounts of free and conjugated dopamine which are excreted in the urine after L-dopa therapy. Thus, part of the variability in the therapeutic responses to L-dopa, as well as the variable side effects, could be due to variations in the conjugation of dopamine which could lead to variations in the amounts of dopamine available to produce physiological effects.

The purpose of the present study was to assess the contribution of the gastrointestinal–portal liver system to the conjugation of L-dopa and its metabolites. Tracer quantities of $^3$H-L-dopa were given both intravenously and orally and the amounts of some of the conjugated and free metabolites excreted in the urine were measured. Since we have previously observed that the proportion of the metabolite which is conjugated decreases as the dose of L-dopa increases [11], we also examined conjugation after therapeutic doses of L-dopa. The hypothesis is that conjugation occurs to a significant extent in the gastrointestinal–portal–hepatic system, and thus it would be expected that conjugation of $^3$H-dopamine derived from intravenous administration of tracer doses of $^3$H-L-dopa would not be affected by large oral doses of L-dopa.

## METHODS

Nine women and twelve men with Parkinsonism participated in at least one phase of the study. Fifteen patients were diagnosed as having idiopathic Parkinsonism, four patients had Parkinsonism secondary to encephalitis lethargica, and two patients had Parkinsonism associated with progressive supra nuclear palsy. The ages of the patients ranged from 49 to 83 yr with a mean age of 66 yr. The patients were withdrawn from their previous medication for at least a week and were admitted to a clinical research ward. The diet was not controlled except that foods containing catecholamines were restricted [14, 15]. Before starting treatment with L-dopa, seven patients received an intravenous infusion in the antecubital vein of 100 μCi (7·7–14 μg) $^3$H-L-dopa (sp. act. 1·4–2·5 Ci/m-mole) dissolved in 1·0 l. of 5% dextrose over a period of 4 hr. Four patients received 200 μCi (15·4–28 μg) $^3$H-L-dopa by mouth in either 100 ml of 0·01

* This investigation was supported by U.S. Public Health Service Research Grant NS-09199 of the National Institutes of Health, and Grant RR-SI from the General Clinical Research Center Program of the Division of Research Resources, National Institutes of Health. A preliminary abstract of this study appeared in *Pharmacologist* **15**, 247 (1973).

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N HCl or 150 ml of 5% dextrose. Fourteen patients then received therapeutic doses of L-dopa which were started at 1·0 g/day in four divided doses. The dosage was increased by 0·5 g/day every 2 days until a dose of 3·0 g/day was reached. While receiving 3·0 g L-dopa/day, six patients received an i.v. infusion of $^3$H-L-dopa as described above. In all phases of the study, 24-hr urine samples were collected beginning at the start of the infusion or upon oral ingestion of L-dopa. The urine was collected in bottles containing 2·0 g sodium metabisulfite as a preservative. Fractions of the 24-hr urine collection were frozen until they could be assayed. An aliquot of the urine was boiled with hydrochloric acid to hydrolyze the conjugated products of dopa and its metabolites [16].

Unlabeled dopa, dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) were isolated from urine samples by Alumina chromatography [17]. Dopa, DA and DOPAC were separated by paper chromatography and assayed by spectrophotofluorometry as described by Sourkes *et al.* [18]. Homovanillic acid (HVA) was assayed by a modification [11] of the procedure of Sato [19] and later by the procedure of Vidi and Bonardi [20]. This latter procedure involves extraction of HVA from acidified urine samples with ethyl acetate, thin-layer chromatography of the extracts, conversion of HVA to a fluorescent derivative and measurements of the derivative by spectrophotofluorometry. Aliquots of the fractions containing each metabolite were used for determination of radioactivity by liquid scintillation spectrometry [21]. The content of each metabolite was corrected for recovery. The recoveries were (mean ± S.E.M.): dopa 72·1 ± 4·0, DA 76·0 ± 4·0, DOPAC 54·6 ± 2·7 and HVA 74·0 ± 4·7 per cent.

*Substances.* L-3,4-Dihydroxyphenylalanine-2,3-$^3$H (sp. act. 1·4 to 2·5 Ci/m-mole) was obtained sterile and pyrogen free from Amersham/Searle Corp., Arlington Heights, Ill. Standard 3-hydroxytyramine hydrochloride (DA), 3,4-dihydroxyphenylacetic acid and homovanillic acid were obtained from CalBiochem, San Diego, Calif. Unlabeled L-dopa was obtained from Schwarz/Mann, Orangeburg, N.Y. L-Dopa which was used in the treatment of patients was obtained as Larodopa® from Hoffmann-La Roche, Inc., Nutley, N.J.

## RESULTS

The urinary excretion of $^3$H-L-dopa and some of its metabolites was measured in 24-hr urine samples after either intravenous or oral administration of the drug. When $^3$H-L-dopa was given intravenously, 83·8 + 5·1 per cent (mean ± S. E. M.) of the total tritium was excreted within the first 24 hr compared to 64·2 ± 5·9 per cent after oral administration. Of the 100 μCi administered intravenously, 44·4 μCi was present as $^3$H-DA, $^3$H-DOPAC and $^3$H-HVA or as conjugates of these metabolites. These metabolites (free and conjugated) amounted to 46·4 μCi/100 μCi when the drug was given orally. When $^3$H-L-dopa was given orally, the major metabolic product was conjugated $^3$H-DA which accounted for 67 per cent of the total amount of $^3$H-DA and its metabolites excreted within the first 24 hr (Fig. 1). When $^3$H-L-dopa was given intravenously, $^3$H-HVA and $^3$H-DA were the major metabolites of those measured and

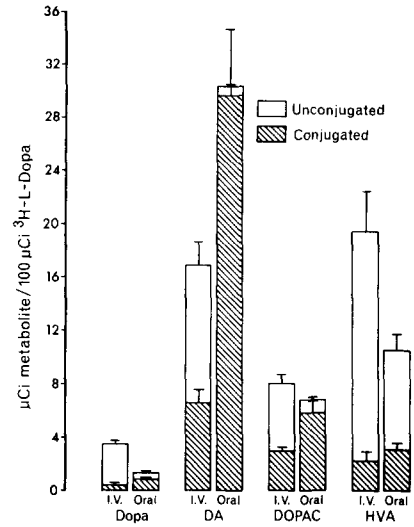


Fig. 1. Urinary excretion of tracer quantities of $^3$H-L-dopa and its metabolites after oral or intravenous administration. The values represent the mean ± S. E. M. of seven values after i.v. administration and four values after oral administration of $^3$H-L-dopa.

they were excreted in approximately equal amounts. Conjugated products of all four compounds were excreted in larger amounts after oral as compared to intravenous administration. The three catechols, dopa, DA and DOPAC, were, in general, conjugated to a greater extent than HVA with either route of administration of $^3$H-L-dopa.

In order to determine if large amounts of L-dopa, such as those used in the treatment of Parkinson's disease, were metabolized differently from trace quantities of $^3$H-L-dopa, the urinary excretion of L-dopa

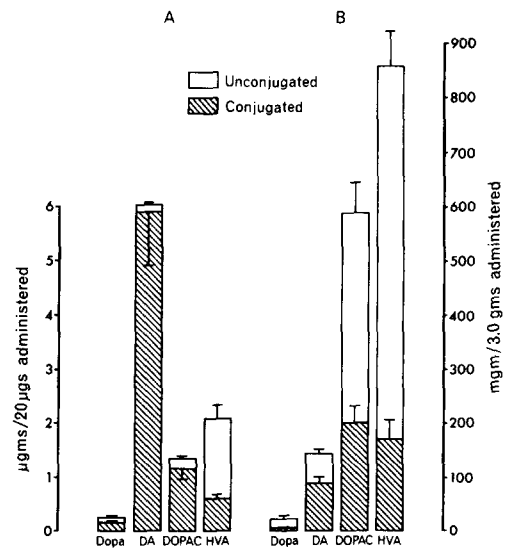


Fig. 2. Comparison of the urinary excretion of dopa and its metabolites after the oral administration of tracer quantities of $^3$H-L-dopa (30 μg) (A) with the excretion of the metabolites after large therapeutic oral doses of L-dopa (3·0 g/day) (B). The values represent the mean ± S. E. M. of four values after the administration of $^3$H-L-dopa and fourteen values after the adminstration of 3·0 g/day of L-dopa.

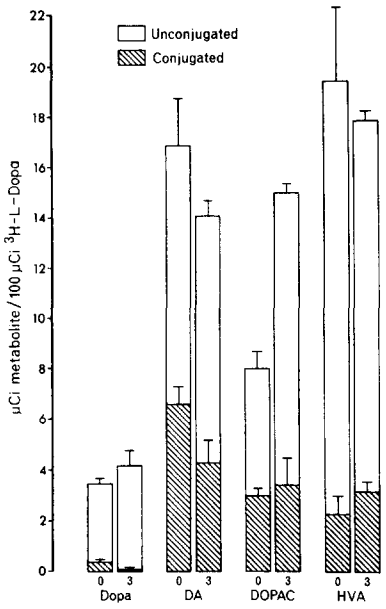


Fig. 3. Comparison of the urinary excretion of $^3$H-L-dopa and its metabolites after intravenous administration of tracer amounts of $^3$H-L-dopa (30 μg) in the presence and absence of 3·0 g/day of L-dopa given orally. The values represent the mean ± S. E. M. of six values in the presence of 3·0 g/day of L-dopa and seven values in the absence of oral L-dopa.

Table 1. Per cent conjugation of L-dopa and metabolites from L-dopa administered orally*

| Dose of L-dopa | Metabolites | | | |
|---|---|---|---|---|
| | Dopa | DA | DOPAC | HVA |
| $^3$H-L-dopa (30 μg) (3) | 76·6 ± 4·4 | 97·3 ± 0·9 | 85·4 ± 3·9 | 30·9 ± 3·2 |
| L-dopa (3·0 g) (14) | 25·6 ± 6·5 | 61·1 ± 3·8 | 24·4 ± 2·5 | 19·8 ± 3·4 |

* Each value is the mean ± S. E. M. of the number of determinations in parentheses.

and some of its metabolites was measured after oral administration of 3·0 g/day of L-dopa (Fig. 2). When large quantities of L-dopa were given, the two deaminated metabolites of DA (DOPAC and HVA) were the primary products of dopa metabolism. This is in contrast to the metabolism of tracer quantities of $^3$H-L-dopa in which conjugated DA is the predominant metabolite. Conjugation of all the metabolites is less after 3·0 g L-dopa as compared to conjugation of 30 μg oral L-dopa (Table 1). This effect is most marked for dopa and DOPAC.

Similar experiments were conducted to determine if large oral doses of L-dopa affected the metabolism of $^3$H-L-dopa given intravenously (Fig. 3). The metabolism of intravenous $^3$H-L-dopa was less affected by 3·0 g oral L-dopa with the exception of increased excretion of free $^3$H-DOPAC in the presence of 3·0 g/day of oral L-dopa. In particular, conjugation of $^3$H-L-dopa and its $^3$H-metabolites was less affected by therapeutic doses of L-dopa when the $^3$H-L-dopa was given intravenously than when L-dopa was given orally (compare Tables 1 and 2).

Table 2. Per cent conjugation of L-dopa and metabolites from 100 μCi $^3$H-L-dopa administered intravenously*

| Dose of oral L-dopa (g) | Metabolites | | | |
|---|---|---|---|---|
| | Dopa | DA | DOPAC | HVA |
| 0 (7) | 10·9 ± 2·4 | 39·5 ± 2·2 | 39·5 ± 4·5 | 15·0 ± 5·5 |
| 3·0 (6) | 2·4 ± 2·0 | 29·9 ± 5·4 | 21·2 ± 5·8 | 17·4 ± 1·7 |

* Each value is the mean ± S. E. M. of the number of determinations in parentheses.

## DISCUSSION

When tracer quantities of $^3$H-L-dopa were given either orally or intravenously, about 45 per cent of the administered dose was collected in the urine as $^3$H-DA or as metabolites of $^3$H-DA within the first 24 hr. This indicates that the decarboxylation of $^3$H-dopa to $^3$H-DA was approximately the same when the drug was given by the two routes. The subsequent metabolism of $^3$H-DA, however, was markedly dependent upon the route by which $^3$H-L-dopa was given. Conjugation of $^3$H-DA with sulfuric acid [11–13, 22] is the primary metabolic pathway of $^3$H-DA derived from orally administered $^3$H-L-dopa, while *O*-methylation and deamination of $^3$H-DA occur to a greater degree when the $^3$H-L-dopa is given intravenously. Other studies have indicated that plasma levels of free DA are lower when it is derived from oral dopa than when it is derived from dopa given intravenously [8, 23, 24]. This is consistent with the concept that conjugation of newly formed DA is a primary route of metabolism when L-dopa is given orally. Other catecholamines such as epinephrine [25] and isoproterenol [26] are also conjugated to a greater degree when they are given orally as compared to parenteral administration. This suggests that, regardless of alternate routes of metabolism, conjugation of catecholamines predominates when given orally. This is obviously not the case for L-dopa, since decarboxylation occurs to a much greater extent than conjugation when this catechol amino acid is given either orally or parenterally. Since phenol sulfotransferase is present in the intestine and liver in relatively large amounts [27, 28], the predominance of conjugation of catecholamines when given orally suggests that sulfate conjugation, under these conditions, occurs primarily in the gastrointestinal tract and possibly in the liver due to hepatic transport of dopamine through the hepatic–portal system.

It was also observed in the present study that large doses of L-dopa result in decreased conjugation of DA. This confirms our earlier observation [11] that therapeutic dosages of L-dopa result in reduced conjugation of dopa and its metabolites. This reduction in conjugation is proportional to the dose of L-dopa and is probably due to an overloading of the phenol sulfotransferase, although it is not related to the total pool of inorganic sulfate, since after even very high doses of L-dopa, approximately 50 per cent of urinary sulfate remains in the inorganic form [11]. The observation that high doses of L-dopa result in a greater effect on conjugation when L-dopa is given orally than when $^3$H-L-dopa is given intravenously is additional evidence that conjugation takes place in the gastrointestinal–hepatic system, since the amounts of phenolic substances present in the gastrointestinal tract would be much higher than in other regions

of the body. The small degree of decreased conjugation of intravenous $^3$H-L-dopa and some of its metabolites which does occur after oral administration of 3·0 g L-dopa might also take place in the gastro intestinal–hepatic system, since intravenous administration of $^3$H-L-dopa in rats leads to an accumulation of $^3$H-L-dopa and $^3$H-DA in the gastrointestinal tract [24, 29, 30]. That is, the conjugation of intravenously administered $^3$H-L-dopa may take place in the gastrointestinal tract and thus one would expect conjugation to be somewhat suppressed by 3·0 g/day of L-dopa given orally.

Since the urinary excretion of the 3-sulfate isomer of DA predominates over that of the 4-sulfate isomer in Parkinsonian patients treated with L-dopa [22], it is apparent that 3-*O*-methylation and 3-sulfate conjugation compete for the 3-hydroxyl group of DA. This is consistent with observations in the present study. Under the conditions in which conjugation was not a major pathway for DA metabolism, such as when $^3$H-L-dopa was given intravenously or when conjugation was suppressed by large doses of oral L-dopa, the amounts of urinary HVA were markedly increased. Thus, it is apparent that *O*-methylation of catechol and phenolic substances should be viewed in light of their ability to be conjugated, since these two enzymatic processes can both be involved at the same site on the substrate molecule.

The results of the present study on the metabolism of an intravenous infusion of $^3$H-L-dopa in Parkinsonian patients agree quite well with the results of Goodall and Alton [10] in which $^{14}$C-L-dopa was infused into healthy normal subjects. This suggests that the metabolism of intravenously administered $^3$H-L-dopa is not aberrant in Parkinsonian patients. The importance of differences in the metabolism of orally and intravenously administered $^3$H-L-dopa in Parkinsonian patients may be that, when conjugation is a minor pathway, there is more free DA to exert toxic side reactions. This would occur when $^3$H-L-dopa is given intravenously to patients in which conjugation after orally administered L-dopa is much less than normal or in patients who are particularly susceptible to decreased conjugation by the large therapeutic doses of L-dopa. Decreased conjugation in the presence of large therapeutic doses of L-dopa may also be a factor involved in achieving the necessary blood levels of L-dopa or brain levels of DA for maximum therapeutic effect.

*Acknowledgements*– The technical assistance of Robert Arnold and Amelia Marlowe is gratefully acknowledged. We thank Hoffmann-La Roche, Nutley, N.J. for the generous contribution of L-dopa (Larodopa®) which was given to the patients.

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