14. B. J. McDonald and W. A. Thompson, *Biometrika* **54**, 487 (1967).
15. S. Udenfriend, *Fluorescence Assay in Biology and Medicine*, p. 132. Academic Press, New York (1962).
16. R. Laverty and K. M. Taylor, *Analyt. Biochem.* **22**, 269 (1968).
17. J. T. Coyle, *Biochem. Pharmac.* **21**, 1935 (1972).
18. H. O. Pappas and D. M. Purpura, *Fine Structure of Cerebral Synapses*, p. 21. Raven Press, New York (1969).
19. T. Hokfelt, G. Johnson and P. Lidbrinke, *Brain Res.* **22**, 147 (1970).
20. J. P. Bennett, W. Logan and S. H. Snyder, *J. Neurochem.* **21**, 1533 (1973).
21. L. L. Iversen, *Br. J. Pharmac. Chemother.* **41**, 571 (1971).
22. S. H. Snyder, A. B. Young, J. P. Bennett and A. H. Mulder, *Fedn Proc.* **32**, 2039 (1973).
23. L. L. Iversen and M. J. Neal, *J. Neurochem.* **15**, 1141 (1968).
24. G. Levi and M. Raiteri, *Brain Res.* **57**, 165 (1973).
25. L. Sokoloff, in *Regional Neurochemistry* (Eds. S. S. Kety and J. Elkes), pp. 107–17. Pergamon Press, New York (1961).
26. H. Goldman and L. A. Sapirstein, *Am. J. Physiol.* **224**, 122 (1973).
27. V. Nair, D. Palm and L. J. Roth, *Nature, Lond.* **188**, 497 (1960).
28. W. J. Nicklas and S. Berl, *Brain Res.* **61**, 343 (1973).
29. J. R. Cooper, F. E. Bloom and R. H. Roth, *The Biochemical Basis of Neuropharmacology*, 2nd Ed., p. 106. Oxford University Press, London (1974).
30. J. A. Romero, J. P. Chalmers, K. Cottman, L. D. Lytle and R. J. Wurtman, *J. Pharmac. exp. Ther.* **180**, 277 (1972).
31. L. A. Wade and R. Katzman, *Am. J. Physiol.* **228**, 352 (1975).

Biochemical Pharmacology, Vol. 25, pp. 1679–1681. Pergamon Press, 1976. Printed in Great Britain.

Effect of carbidopa on the metabolism of L-dopa in the pigtail monkey*

(*Received* 6 *November* 1975; *accepted* 16 *January* 1976)

Carbidopa [L-2-hydrazine-α-methyl-β-(3,4-dihydroxy phenyl) propionic acid (MK-486)] is an inhibitor of extracerebral dopa decarboxylase which has been used in combination with L-dopa in the therapy of Parkinson's disease [1–3]. Inhibition of peripheral dopa decarboxylase allows greater quantities of L-dopa to reach the brain where it is then decarboxylated to dopamine, the active metabolite. We have examined the effect of carbidopa on the metabolism of [^{3}H]L-dopa administered intravenously to the pigtail monkey. In a preliminary study [4], the excretion of [^{3}H]dopamine in the urine was markedly reduced by carbidopa, while the urinary excretion of [^{3}H]homovanillic acid, a major dopamine metabolite, was not decreased. It was suggested that, in the presence of carbidopa, L-dopa is converted to homovanillic acid through a pathway which does not involve decarboxylation to dopamine but may involve a sequence of reactions which include *O*-methylation, transamination and oxidative decarboxylation. Recently, a shift in the metabolism of oral L-dopa from decarboxylation to transmination has been reported [5] in Parkinsonian patients treated with carbidopa. Since the sulfate conjugates of dopamine can represent major metabolites of L-dopa in Parkinsonian patients [6–9], the excretion of dopamine 3-*O*-sulfate and dopamine 4-*O*-sulfate was also determined in control and carbidopa-treated monkeys.

Decarboxylation of L-dopa was measured *in vivo* in the unanesthetized monkey in order to determine the effectiveness of carbidopa as an inhibitor of dopa decarboxylase under the conditions of this study. The procedure used by Hansson and Clark [10] for mice was modified for use in monkeys. A pigtail monkey (4.55 kg) was injected in the internal saphenous vein with 0.25 μCi (13.8 μg) of [^{14}C]carboxy-labeled L-dopa (3.56 mCi/m-mole). Exhaled $^{14}CO_2$ was trapped in a solution of 27% phenylethylamine, 27% methanol and 46% toluene at 10-min intervals for 2 hr. Radioactivity was determined in each of the 10-min fractions by liquid scintillation spectrometry [11]. In a separate experiment, more than 2 weeks later, carbidopa, 360 mg/kg, was administered orally by nasal gastric tube in a suspension of 0.5% methylcellulose (adjusted to pH 4.2) 1 hr prior to the injection of [^{14}C]L-dopa. The effect of carbidopa can be seen in Fig. 1. In the untreated monkey, 38 per cent of the radioactivity was exhaled in the first 60 min. During the same interval, only 11 per cent of the label was exhaled by monkeys receiving the decarboxylase inhibitor and, therefore, a 71 per cent inhibition of enzyme activity was achieved. Similar results were obtained when the experiment was repeated in a rhesus monkey (3.7 kg). Similar inhibition of dopa decarboxylase was also seen when pigtail monkeys were pretreated with the inhibitor 2 and 3 hr prior to [^{14}C]L-dopa.

The effect of carbidopa was then determined on the metabolism of [3-^{14}C]L-dopa (labeled on the β-carbon) administered intravenously to pigtail monkeys. A Foley catheter was placed in the urinary bladder of each of three female pigtail monkeys (4.2 to 5.7 kg) and urine was collected continuously. Each monkey was injected with 10–25 μCi (100–250 μg) of [3-^{14}C]L-dopa (sp. act. 20–40 mCi/m-mole) into the internal saphenous vein. Not less than 2 weeks later, the monkeys were treated with carbidopa as described above and the injection with [3-^{14}C]L-dopa was given 2.5 hr later. Carbidopa, 360 mg/kg, was given again 12 hr after the initial drug treatment. Each monkey received [^{14}C]L-dopa four times, twice as a control and twice after carbidopa pretreatment. Urine samples were collected for 24 hr after the injection of [3-^{14}C]L-dopa in bottles containing 200 mg sodium metabisulfite. The metabolites of L-dopa were measured with a liquid chromatography flow monitoring system described by Goodall and Alton [12]. The acidic metabolites, dihydroxyphenylacetic acid, homovanillic acid, vanillactic acid, dopamine 3-*O*-sulfate, dopamine 4-*O*-sulfate and methoxytyramine sulfate, were eluted from a 0.9 by 35 cm Dowex 1 anion exchange column. The effluent from the ion

* This investigation was supported by U.S. Public Health Service Research Grant NS-09199 of the National Institutes of Health.

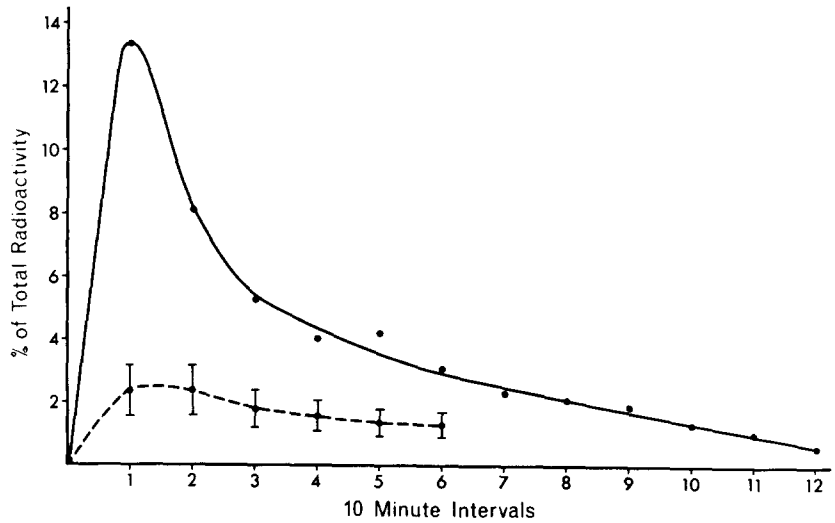

Fig. 1. Effect of 360 mg/kg of carbidopa on the decarboxylation of i.v. [^{14}C]carboxy-labeled-L-dopa. Results are expressed as the per cent of $^{14}CO_2$ exhaled/10-min interval of the total radioactivity which waa injected. Carbidopa was administered 1 hr prior to [^{14}C]L-dopa and the values are the mean ± S.E.M. of three determinations. Key: ●——●, control; ●- - -●, carbidopa.

exchange column containing the metabolites was then passed through an anthracene flow cell for determination of radioactivity of each metabolite. In some cases, the radioactivity was too small to be detected in the flow cell. Under these conditions, 14.5-ml samples of the eluate were collected in a fraction collector and the radioactivity was determined in each sample by liquid scintillation spectrometry.

Since homovanillic acid and vanillactic acid co-chromatographed in this system, the values of these two metabolites were determined by a separate procedure. Six ml of urine was adjusted to pH 1 with 6 N HCl. The two metabolites were extracted three times into 10 ml ethyl acetate. The 30 ml of ethyl acetate was washed two times with 10 ml of 10% $NaHCO_3$. The $NaHCO_3$ solution was adjusted to pH 1 with 6 N HCl, and the metabolites were again extracted three times into 10 ml ethyl acetate. The ethyl acetate extract was then evaporated to dryness on a rotary evaporator and 0.5 ml methanol was added to the flask. The methanol extract was applied to a Silica

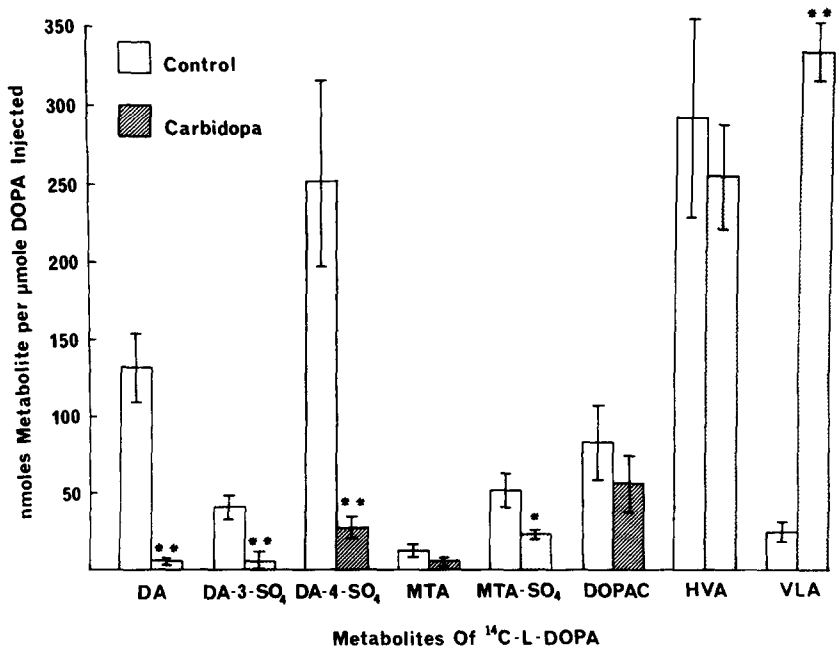

Fig. 2. Effect of carbidopa on the metabolism of [3-^{14}C]L-dopa. Values are expressed as the mean ± S. E. M. from 24-hr urine samples in duplicate experiments in each of three monkeys. Significance of the differences between control and carbidopa-treated monkeys was determined by Student's *t*-test. The single asterisk (*) indicates $P < 0.05$; the double asterisk (**) indicates $P < 0.01$. Abbreviations: DA, dopamine; DA-3-SO_4, dopamine 3-*O*-sulfate; DA-4-SO_4, dopamine 4-*O*-sulfate; MTA, methoxytyramine; MTA-SO_4, methoxytyramine 4-*O*-sulfate; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; and VLA, vanillactic acid.

gel GF plate (250 μm). The metabolites were chromatographed in a solvent system consisting of 31 ml benzene, 18 ml of gacial acetic acid and 0.8 ml water. The R_f for homovanillic acid was 0.62 and the R_f for vanillactic acid was 0.28. The spots were visible with u.v. light. The spots were removed and eluted with 4 ml of 0.05 M Tris buffer (pH 8.6). Radioactivity was determined in each eluate by liquid scintillation spectrometry.

The values of the metabolites obtained in 24-hr urine samples are shown in Fig. 2. In control experiments, the eight metabolites measured contained 89 per cent of the total radioactivity which was injected, while in animals pretreated with the decarboxylase inhibitor only 62 per cent of the radioactivity was accounted for by these metabolites. This decrease in radioactivity could be due to increased formation of metabolites which are excreted at slower rates after carbidopa treatment. The excretion of dopamine, dopamine 3-*O*-sulfate, dopamine 4-*O*-sulfate and methoxytyramine sulfate was markedly decreased after treatment with carbidopa. Homovanillic acid, 3,4-dihydroxyphenylacetic acid and methoxytyramine excretion were not significantly lowered. Vanillactic acid, which is a stable intermediate formed following the transamination of dopa or 3-*O*-methyldopa, increased approximately 14-fold after inhibition of dopa decarboxylase, and became the major metabolite measured in the samples.

Vanillactic acid is clearly a minor metabolite in monkeys not receiving carbidopa and represents only 1 per cent of the L-dopa dose excreted in Parkinsonian patients [13]. Because it is the major metabolite measured after carbidopa pre-treatment, transamination of L-dopa or 3-*O*-methyldopa [14] becomes a major pathway of metabolism. In studies of the metabolism of [^{14}C]vanillactic acid in rats [15], homovanillic acid accounted for 26 per cent of the radioactivity excreted and a major amount of [^{14}C]methoxytyramine was formed. Therefore, the further metabolism of vanillactic acid to metabolites of L-dopa normally formed after decarboxylation could be at least partially responsible for the failure of homovanillic acid to decrease significantly after decarboxylase inhibition. Confirmation of this suggestion could be obtained by the administration of labeled vanillactic acid and measuring the formation of labeled homovanillic acid both before and after the administration of carbidopa.

3,4-Dihydroxyphenylacetic acid and homovanillic acid levels have been reported to be reduced significantly in Parkinson patients pretreated with carbidopa [5, 16]. However, the dose (360 mg/kg) of carbidopa used in the present study is much larger than the dose given to patients with Parkinson's disease. In the studies by Messiha *et al.* [16, 17], when the pretreatment dose of 100 mg carbidopa was increased to 100 mg three times daily for 7 days prior to L-dopa administration, the reduction in urinary homovanillic acid was much smaller. Thus, it is possible that at higher dose levels transamination occurs to a greater degree and that the two acid metabolites are formed by this pathway.

Much of the dopamine excreted by Parkinsonian patients is conjugated as the sulfate [7, 8]. Dopamine is conjugated in the 3 position or the 4 position and, in Parkinsonian patients receiving [^{3}H]dopa either intravenously or orally dopamine 3-*O*-sulfate predominates [18, 19]. We have found that, in monkeys given [^{14}C]L-dopa intravenously, dopamine 4-*O*-sulfate was one of the major metabolites excreted and was formed in an amount six times that of dopamine 3-*O*-sulfate. It therefore appears that a difference exists in the sulfate conjugation of dopamine in man and monkey but it is unclear what factor or factors are responsible for determining which isomer will predominate because the sites of sulfation and the enzyme kinetics of the sulfotransferases in the two species are not known.

Acknowledgements—We are indebted to Diann Miller and Robert Arnold for their excellent technical assistance. We would also like to thank Merck, Sharp & Dohme for their generous contribution of carbidopa.

Department of Pharmacology, University of Colorado School of Medicine, Denver, Co., 80220, U.S.A.

ROBERT L. BRONAUGH*
GALEN R. WENGER†
DAVID L. GARVER‡
CHARLES O. RUTLEDGE§

REFERENCES

1. G. C. Cotzias, P. S. Papavasliou and R. Gellene, *New Engl. J. Med.* **280.** 337 (1969).
2. D. B. Calne, J. L. Reid, S. D. Vakil, S. Rao, A. Petrie, C. A. Palis, J. Gowler, P. K. Thomas and A. Hilson, *Br. med. J.* **3.** 729 (1971).
3. M. E. Jaffe, *Adv. Neurol.* **2.** 161 (1973).
4. R. L. Bronaugh, G. R. Wenger, D. L. Garver and C. O. Rutledge, *Pharmacologist* **16.** 236 (1974).
5. M. Sandler, R. D. Johnson, C. R. J. Ruthven, J. L. Reid and D. B. Calne, *Nature, Lond.* **247.** 364 (1974).
6. McC. Goodall and H. Alton, *Biochem. Pharmac.* **17.** 905 (1968).
7. McC. Goodall and H. Alton, *J. clin. Invest.* **48.** 2300 (1969).
8. C. O. Rutledge and M. M. Hoehn, *Nature, Lond.* **244.** 447 (1973).
9. R. L. Bronaugh, R. J. McMurtry, M. M. Hoehn and C. O. Rutledge, *Biochem. Pharmac.* **24.** 1317 (1975).
10. E. Hansson and W. G. Clark, *Proc. Soc. exp. Biol. Med.* **111.** 793 (1962).
11. R. J. Ziance, A. J. Azzaro and C. O. Rutledge, *J. Pharmac. exp. Ther.* **182.** 284 (1972).
12. McC. Goodall and H. Alton, *Biochem. Pharmac.* **21.** 2401 (1972).
13. D. B. Calne, F. Karoum, C. R. J. Ruthven and M. Sandler, *Br. J. Pharmac. Chemother.* **37.** 57 (1969).
14. G. Bartholini, I. Kuruma and A. Pletscher, *Br. J. Pharmac. Chemother.* **40.** 461 (1970).
15. G. Bartholini, I. Kuruma and A. Pletscher, *J. Pharmac. exp. Ther.* **183.** 65 (1972).
16. F. S. Messiha, T. H. Hsu and J. R. Bianchine, *Biochem. Pharmac.* **21.** 2144 (1972).
17. J. R. Bianchine, F. S. Messiha and T. J. Preziosi, *Adv. Neurol.* **2.** 101 (1973).
18. W. N. Jenner and F. A. Rose, *Nature, Lond.* **252.** 237 (1974).
19. R. L. Bronaugh, S. E. Hattox, M. M. Hoehn and C. O. Rutledge, *J. Pharmac. exp. Ther.* **195.** 441 (1975).

* Present address: INTERx Research Corp. Lawrence, Kansas 66044.

† Present address: Laboratory of Psychobiology, Harvard Medical School, 25 Shattuck St., Boston, Mass. 02115.

‡ Present address: Illinois State Psychiatric Institute, 1601 West Taylor St., Chicago, Ill. 60612.

§ Present address: Department of Pharmacology and Toxicology, School of Pharmacy, The University of Kansas, Lawrence, Kans, 66045. Address reprints requests to this address.