MOLECULAR PHARMACOLOGY, 15, 108-114

Inhibition of Human and Rat Hepatic Aminotransferase Activity with L-3,4-Dihydroxyphenylalanine by Inhibitors of Peripheral Aromatic Amino Acid Decarboxylase

MARILYN J. WATERHOUSE, YUET CHING CHIA AND G. J. LEES

Department of Biochemistry, University of Auckland, Auckland, New Zealand

(Received March 17, 1978)
(Accepted August 18, 1978)

SUMMARY

WATERHOUSE, MARILYN J., CHIA, YUET CHING, & LEES, G. J. (1979) Inhibition of human and rat hepatic aminotransferase activity with Dopa by inhibitors of peripheral aromatic amino acid decarboxylase. *Mol. Pharmacol.* **15**, 108-114.

Dopa aminotransferase activity in homogenates of human or rat liver was inhibited *in vitro* by MK 486. With the enzyme from rat, inhibition was more marked when α-ketoglutarate was used as the amino group acceptor rather than phenylpyruvate. In concentrations up to 30 μM, NSD 1055 but not Ro 4-4602 also inhibited Dopa aminotransferase. All three drugs inhibited aromatic amino acid decarboxylase more effectively than Dopa aminotransferase. The degree of inhibition of Dopa aminotransferase activity by MK 486 was not affected by the pH of the assay. The inhibition could be overcome by high concentrations of pyridoxal phosphate. *In vivo,* Ro 4-4602, but not MK 486 caused an increase in the hepatic levels of Dopa-α-ketoglutarate and tyrosine-α-ketoglutarate aminotransferase activities in male rats. These drugs had little effect on the corresponding activities in female rats and no significant increase in the aminotransferase activities occurred when phenylpyruvate was used as the amino group acceptor.

INTRODUCTION

In mammalian tissues alternative pathways exist for the metabolism of L-3,4-dihydroxyphenylalanine (Dopa). These include O-methylation, transamination and decarboxylation. Of these pathways, decarboxylation of Dopa by aromatic amino acid decarboxylase (EC 4.1.1.26) is the most important, even after administration of large amounts of additional L-Dopa during the treatment of Parkinsonism (1, 2). More recently Parkinsonism has been treated by a combination of L-Dopa and peripheral decarboxylase inhibitors such as MK 486 (Carbidopa, α-methyl-α-hydrazino-3,4-dihydroxyphenylpropionic acid monohydrate) or Ro 4-4602 (Benserazide, N^1-[DL-seryl]-N^2-[2,3,4]trihydroxybenzylhydrazine). Under these conditions a large proportion of Dopa is metabolized by transamination in man (3) and monkey (4).

Transaminations between Dopa and keto acids in liver have not been studied in great detail, although tyrosine aminotransferase (EC 2.6.1.5) can catalyze such reactions (5). The purpose of this study was to evaluate the effects of peripheral decarboxylase inhibitors on the transamination of keto acids by Dopa *in vitro* and to compare the resulting inhibition with that of aromatic amino acid decarboxylase.

This research was supported by a grant from the Auckland Medical Research Foundation.

MATERIALS AND METHODS

Preparation of homogenates. Sprague-Dawley rats (160-260 g, Middlemore Hos-

0026-895X/79/010108-07$02.00/0

pital, Auckland, New Zealand), were decapitated, and a portion of the liver excised, weighed and homogenized in 9 volumes of glass-distilled water in a ground glass homogenizer. Where indicated 1 ml of liver homogenate was dialyzed overnight at 4° against 1 litre of 0.1 M sodium phosphate buffer (pH 7.0), with and without cysteine (50 mM) and 2-mercaptoethanol (20 mM). When homogenates were dialyzed overnight, rats were killed between 1500 hr and 1700 hr. Otherwise, killing occurred between 1000 hr and 1100 hr. Samples of human liver were obtained by biopsy and immediately placed on ice. The samples were then either stored for 16 hr at −20° or were homogenized in a microhomogenizer and assayed immediately.

Enzyme assays. Aminotransferase activity with Dopa was assayed by measuring the production of [^{14}C]dihydroxyphenylpyruvate from L-[3-^{14}C]Dopa. The reaction mixture contained (unless otherwise indicated) 0.76 μmole N-tris (hydroxymethyl) methylglycine (tricine) (pH 8.5), 32.4 nmole L-[3-^{14}C]Dopa (0.69 μCi/μmole), 5 nmole sodium phenylpyruvate, 0.76 nmole pyridoxal phosphate, and 7 nmole pargyline in a volume of 7 μl. Where indicated sodium phenylpyruvate was replaced by 50 nmole disodium α-ketoglutarate. The enzyme was preincubated for 5 min at 37° with varying concentrations of inhibitor (in a ratio of 1 vol enzyme to 3 vol inhibitor) and 4 μl of this mixture was added to the reaction mixture. (1 μl of liver homogenate contained 20 μg protein.) The reaction was assayed and products analyzed as described previously (6). The assays for Dopa aminotransferase activity (using either α-ketoglutarate or phenylpyruvate) showed linear increases with increasing protein concentration and times of incubation up to 30 min. Thus oxidation of Dopa at the relatively high pH employed is not a problem. MK 486 is also a catechol and could be oxidized at pH 8.5. However, the effectiveness of MK 486 as an inhibitor was the same in assays incubated for 15 min instead of 30 min. All inhibitors were made up in boiled distilled water on the day of use. Inhibition of Dopa aminotransferase activity was markedly reduced with aged solutions of MK 486.

Tyrosine aminotransferase was assayed as described previously (6) using either 50 μmole sodium α-ketoglutarate or 20 μmole sodium phenylpyruvate.

For the assay of aromatic amino acid decarboxylase, the reaction mixture ordinarily contained 1.25 μmole imidazole acetate (pH 6.6), 6.25 nmole ethylenediamine tetraacetic acid (pH 7.0), 1.25 nmole pyridoxal phosphate and 12.5 nmole L-[1-^{14}C]Dopa (5.9 μCi/μmole) in a volume of 7 μl. When the enzyme was preincubated with pyridoxal phosphate (5 min at 37°) no additional pyridoxal phosphate was added to the reaction mixtures. Assay tubes were cooled on ice during the addition of 4 μl of the enzyme/inhibitor mixture described above. The tubes were sealed with rubber serum caps through which was suspended a nickel-chromium wire attached to a glass well containing a piece of filter paper soaked in 10 μl Protosol. After incubation at 37° for 20 min, the tubes were returned to the ice bath while 25 μl of 20% TCA were added through the cap by a Hamilton syringe. The decarboxylase assay was linear with increasing protein concentration and time of incubation up to 20 min. The CO_2 released by the acid was trapped in the Protosol during a further incubation of 3 hr at 37°, or overnight at 4°. A 98% recovery of $^{14}CO_2$ was obtained. The glass wells were removed and placed in 10 ml scintillation fluid containing 5 g 2,5-diphenyloxazole, 0.1 g 1,4-bis[2(5-phenyloxazolyl)]benzene and 5 ml absolute ethanol per litre of toluene. Radioactivity was determined by counting the vials for 10 min in a Packard Tri-carb Liquid Scintillation Spectrometer, Model 3320, with a window setting of 60 to 1000 and a gain of 5%. The counting efficiency for ^{14}C was 83%. All determinations were done in triplicate. The protein concentrations in homogenates of liver were measured by the method of Lowry *et al.* (7). In studies *in vivo,* rats were injected intraperitoneally with 0.9% saline (for the controls) or with drugs at the appropriate concentration in 0.9% saline.

Materials. L-[1-^{14}C]Dopa and L-[3-^{14}C]-Dopa were obtained from The Radiochemical Centre, Amersham, Bucks., England. Protosol was obtained from Consolidated

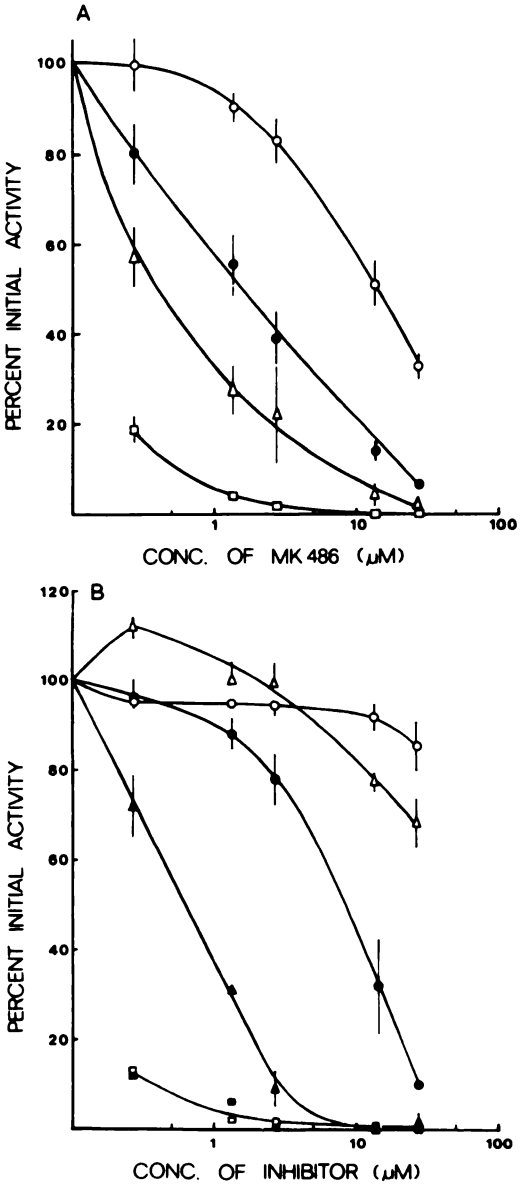

FIG. 1. *Inhibition of Dopa aminotransferase activity and aromatic amino acid decarboxylase in homogenates of human and rat liver by inhibitors of aromatic amino acid decarboxylase.*

The reactions were carried out as described in METHODS. The final concentrations of inhibitors in the reaction mixtures are plotted on the abscissa (concentrations of inhibitors in the preincubation mixtures were 2.75 times higher). Initial activity was determined using homogenates preincubated with water in place of inhibitor. Error bars give the SEM of 3–5 determinations. (A) Inhibition of enzymic activities by MK 486. Dopa aminotransferase activity with undialyzed homogenates of rat liver at pH 8.2 using phenylpyruvate (○), or α-ketoglutarate (●) as acceptors, or human liver homogenates using α-ketoglutarate as acceptor (△). Aromatic amino acid decarboxylase activity at pH 6.6 in undialyzed homogenates of rat liver (□). (B) Inhibition of enzymic activities by Ro 4-4602 or NSD 1055. Dopa-phenylpyruvate aminotransferase activity after preincubation with Ro 4-4602 (○) or NSD 1055 (●). Dopa-α-ketoglutarate aminotransferase activity after preincubation with Ro 4-4602 (△) or NSD 1055 (▲). Aromatic amino acid decarboxylase activity after preincubation with Ro 4-4602 (□) or NSD 1055 (■).

Nucleonics Pty. Ltd., Waterloo, N.S.W., Australia. All other compounds were of the highest purity commercially available. The inhibitors MK 486 (Carbidopa) and Ro 4-4602 (Benserazide) were kindly donated by Frosst, Merck, Sharp & Dohme (New Zealand) Ltd., Auckland, and Roche Products Pty. Ltd., Auckland, New Zealand, respectively. 3-Hydroxy-4-bromobenzyloxyamine dihydrogen phosphate (NSD 1055) was purchased from Smith & Nephews (Herts., England).

RESULTS

Inhibition of Dopa aminotransferase activity and aromatic amino acid decarboxylase by MK 486. The inhibition of aminotransferase activity with Dopa and aromatic amino acid decarboxylase by MK 486 *in vitro* was examined at pH values at which the enzymes showed optimum activity (8.2 and 6.6 respectively) (Fig. 1a), and also at a common pH of 7.1. Although enzymic activities were lower at the common pH, the relative effectiveness of MK 486 as an inhibitor of the two enzymes was unchanged. Over the concentration range used MK 486 was a stronger inhibitor of the decarboxylase than the aminotransferase. At a final concentration of MK 486 of 0.27 μM, Dopa-phenylpyruvate aminotransferase activity from rat liver was not inhibited while aromatic amino acid decarboxylase was inhibited by approximately 80%. A higher concentration of MK 486 of 27 μM resulted in a 70% inhibition of this aminotransferase.

MK 486 was 7-fold more effective (ED_{50} = 2.1 ± 0.5 μM, n = 4) as an inhibitor of rat liver Dopa aminotransferase activity when α-ketoglutarate (50 nmole) replaced phenylpyruvate (5 nmole) as the keto acid (Fig.

1a). The difference in effectiveness of MK 486 as an inhibitor was not due to a preferential reaction of MK 486 with either of the keto acids. Preincubation of MK 486 with the keto acids (30 min at 37°) did not alter the inhibitory properties of MK 486. However activity with α-ketoglutarate was only about half that obtained with phenylpyruvate (Table 1). On the other hand, Dopa aminotransferase activity in human liver was about 8-fold more active with α-ketoglutarate (197 ± 39 nmole × hr^{-1} × mg $protein^{-1}$, n = 5) than with phenylpyruvate (22.8 ± 6.4 nmole × hr^{-1} × mg $protein^{-1}$, n = 4). It was also more sensitive to inhibition by MK 486 than the enzyme from rat liver (ED_{50} = 0.40 ± 0.10 μM, n = 3; Fig. 1a). Dopa-α-ketoglutarate aminotransferase activity in homogenates of human liver was unstable at 0°. Between 36% and 46% of its initial activity was lost within 1 hr at 0°. Dopa aminotransferase activity in rat liver was comparatively stable over this period when assayed with either phenylpyruvate (99.4 ± 4.5%, n = 6, of initial activity) or with α-ketoglutarate (85.0 ± 8.3%, n = 6, of initial activity).

The inhibition of aminotransferase activity by MK 486 did not appear to be due to a reaction between MK 486 and pyridoxal phosphate. Dialysis of homogenates of rat liver against phosphate buffer, or against phosphate-cysteine buffer, did not alter the effectiveness of MK 486 as an inhibitor of Dopa-phenylpyruvate aminotransferase activity. Its effectiveness was also unaltered if pyridoxal phosphate was eliminated from the reaction mixtures. On the other hand, at high concentrations of pyridoxal phosphate (0.35 mM) the inhibition caused by 27 μM MK 486 could largely be reversed (from 72% to 14% inhibition).

Kinetics of the inhibition of Dopa aminotransferase activity by MK 486. Dopa aminotransferase activity in homogenates of rat liver exhibited K_m values for Dopa of 4.6 ± 0.4 mM (n = 5) and 1.7 ± 0.4 mM (n = 5) when assayed in the presence of 0.4 mM phenylpyruvate or 2 mM α-ketoglutarate respectively (Fig. 2). Inhibition by MK 486 was competitive with Dopa when the aminotransferase was assayed with phenylpyruvate (K_i = 5.3 μM) (Fig. 3). Homogenates of rat liver pretreated with 75 μM MK 486 recovered 99% and 86% of the Dopa-phenylpyruvate and Dopa-α-ketoglutarate

TABLE 1

Effect of MK 486 and Ro 4-4602 in vivo on DOPA and tyrosine aminotransferase in male and female rats

Rats were injected intraperitoneally with 0.9% saline, MK 486 (50 mg/kg), or Ro 4-4602 (50 mg/kg) at 0900 to 1000 hr and sacrificed 5 hr later. The activity of the aminotransferases was assayed as described in Methods except that the reaction volume was 13 μl. Values represent means ± SEM for the number of determinations given in parentheses.

Drug injected	Aminotransferase activity			
	Female		Male	
Keto acid acceptor:	α-Ketoglutarate	Phenylpyruvate	α-Ketoglutarate	Phenylpyruvate
	nmole × hr^{-1} × mg $protein^{-1}$			
DOPA aminotransferase activity				
Saline (control)	35.3 ± 4.2 (22)	142 ± 10 (15)	59 ± 11 (15)	132 ± 10 (12)
MK 486	43.3 ± 8.6 (8)	139 ± 6 (4)	—	155 ± 12 (4)
Ro 4-4602	62.5 ± 6.8 (12)*	140 ± 7 (6)	165 ± 25 (14)**	131 ± 2 (13)
Tyrosine aminotransferase activity				
Saline (control)	268 ± 21 (20)	119 ± 8 (15)	414 ± 30 (14)	106 ± 5 (14)
MK 486	291 ± 34 (8)	139 ± 6 (4)	263 ± 24 (8)**	74 ± 3 (4)**
Ro 4-4602	283 ± 36 (8)	111 ± 4 (6)	725 ± 72 (14)**	121 ± 6 (14)

* $p < 0.01$ vs. controls (two-tailed Student's t).

** $p < 0.001$ vs. controls (two-tailed Student's t).

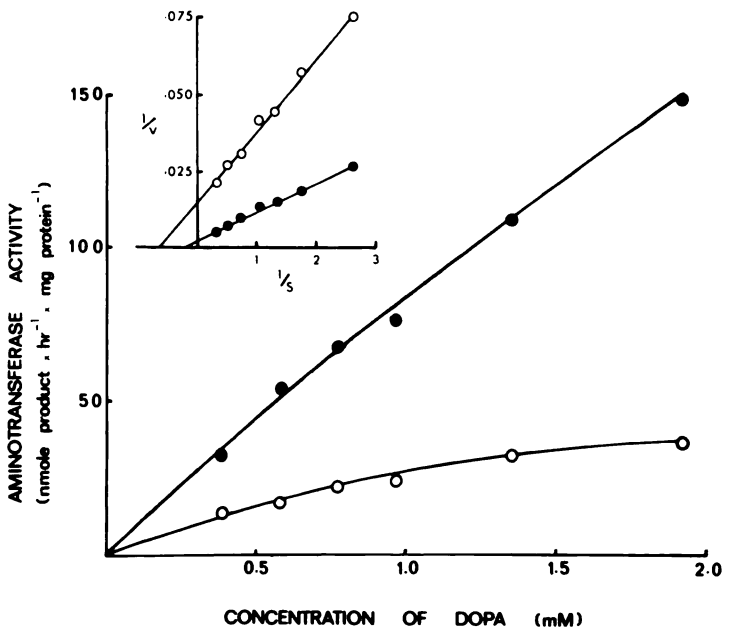

FIG. 2. *Dopa aminotransferase activity in homogenates of rat liver plotted as a function of Dopa concentration.*

The reactions were carried out as described in METHODS. Activity in the presence of 2 mM α-ketoglutarate (○) or 0.4 mM phenylpyruvate (●). Inset: Lineweaver-Burk plot of the results. Lines of best-fit were drawn after linear regression analysis.

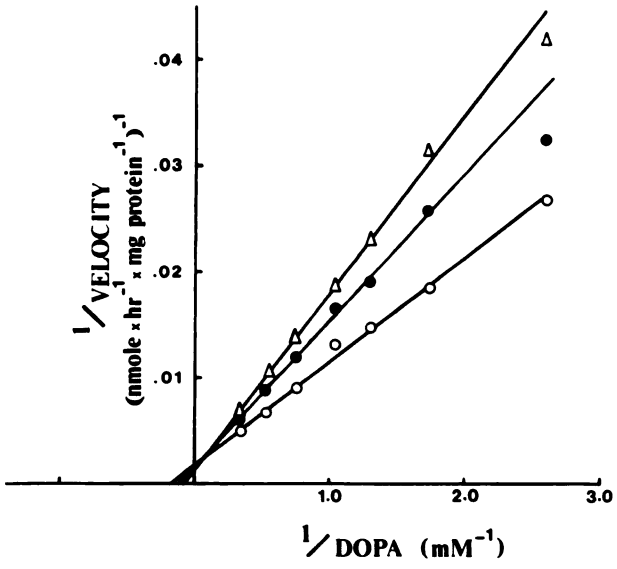

FIG. 3. *Inhibition of Dopa aminotransferase activity by MK 486 plotted as a function of Dopa concentration.*

Results are plotted according to the method of Lineweaver and Burk. Lines of best-fit were drawn after linear regression analysis. The reactions were carried out as described in METHODS except that MK 486 was not preincubated with rat homogenates. Activity with 0.4 mM phenylpyruvate (○) and 0.4 mM phenylpyruvate with 1.5 μM (●) and 3 μM (△) MK 486.

aminotransferase activities respectively when dialyzed against 0.1 M sodium phosphate buffer (pH 7).

Effect of Ro 4-4602 and NSD 1055 on enzymic activity. Two other inhibitors of aromatic amino acid decarboxylase were tested as inhibitors of rat liver Dopa aminotransferase activity. The enzyme was little affected by Ro 4-4602 at concentrations up to 30 μM, whereas NSD 1055 was as effective as MK 486 (Fig. 1b). Both drugs were equally effective as MK 486 as inhibitors of aromatic amino acid decarboxylase. Ro 4-4602 was also a poor inhibitor of human liver Dopa-α-ketoglutarate aminotransferase activity (15% inhibition by 27 μM Ro 4-4602).

Effects of MK 486 and Ro 4-4602 on enzyme levels in vivo. The effects of MK 486 and Ro 4-4602 *in vivo* on Dopa aminotransferase activity also were determined. Within 5 hr after injection, Ro 4-4602 increased the levels of Dopa-α-ketoglutarate aminotransferase activity nearly 3-fold in male rats. A smaller effect was observed in female rats (Table 1). Activity with phenylpyruvate did not show a corresponding increase. MK 486 had little effect on either activity in male or female rats. The pattern of the changes induced by Ro 4-4602 and MK 486 also was similar when tyrosine aminotransferase activities were assayed in the presence of α-ketoglutarate or phenylpyruvate except that a small but significant MK 486-induced decrease was observed in male rats (Table 1). The lack of an effect of MK 486 or Ro 4-4602 on Dopa-phenylpyruvate aminotransferase activity was not due to a slower response *in vivo*. Over a 24 hr period MK 486 or Ro 4-4602 had little effect on this activity in male rats.

DISCUSSION

Metabolism of Dopa by transamination is a potential fate of Dopa administered orally during the treatment of Parkinsonism. However, this does not appear to occur substantially in humans (1, 2), unless concomitantly treated with peripheral decarboxylase inhibitors (3).

Hepatic transaminations by Dopa are poorly characterized. At least part of the activity would appear to be due to tyrosine aminotransferase activity since purified tyrosine aminotransferase from rat liver catalyzes transaminations by both Dopa and tyrosine (5, 8). The tyrosine aminotransferase has an apparent K_m value for Dopa of 2.6 mM, using α-ketoglutarate as the amino group acceptor (8). A similar result was obtained in this investigation but with phenylpyruvate the K_m value for Dopa was approximately doubled. In comparison aromatic amino acid decarboxylase has a K_m value for Dopa of 0.4 mM (9). On the basis of the relative K_m values for Dopa, decarboxylation rather than transamination would be favored. This may be why transaminations by Dopa are significant only in the presence of decarboxylase inhibitors *in vivo*.

In this study MK 486 was tested as an inhibitor of the transamination of both phenylpyruvate and α-ketoglutarate by Dopa. Absolute activity in homogenates from rat liver was lower with α-ketoglutarate and was more sensitive to inhibition by MK 486. However, transamination of either keto acid by Dopa was less sensitive to inhibition than was aromatic amino acid decarboxylase. Dopa-α-ketoglutarate aminotransferase activity in homogenates of human liver was more sensitive to inhibition by MK 486 than the corresponding activity in homogenates of rat liver.

It is difficult to reconcile this inhibition of human and rat Dopa-α-ketoglutarate aminotransferase by MK 486 with the observation that *in vivo*, transamination of Dopa does not occur to a significant extent in the absence of MK 486 in humans (1–3) or rats (10). A possible explanation is that the inhibition of the aminotransferase is transitory, leading to a subsequent shunting of Dopa through this pathway while the decarboxylase is still inhibited.

MK 486 appears to be a reversible inhibitor of Dopa aminotransferase. In contrast inhibition of aromatic amino acid decarboxylase appears to be pseudo-irreversible (11) due to potent binding of the inhibitor to the enzyme (12).

The lower inhibition of Dopa aminotransferase activity by Ro 4-4602 may be due partially to a masking of the hydrazino group by a seryl residue in this compound.

Fellman *et al.* (13) have shown that higher concentrations of Ro 4-4602 than used in this investigation (170 μM) inhibited tyrosine-α-ketoglutarate aminotransferase by 50%.

The differential effects of Ro 4-4602 *in vivo* on Dopa and tyrosine aminotransferase activities assayed with α-ketoglutarate or phenylpyruvate raises the possibility that at least two aminotransferases exist in rat liver which have differing specificities for the keto acid acceptor. This hypothesis is supported by the species difference in the ability of human and rat aminotransferases to use α-ketoglutarate or phenylpyruvate as acceptors of the amino group of Dopa. The effects of Ro 4-4602 and MK 486 *in vivo* on tyrosine-α-ketoglutarate aminotransferase activity are in general agreement with other workers. Thus, David *et al.* (14) found that Ro 4-4602 increased the levels of tyrosine aminotransferase by up to 180% in the liver of the rat. However, Deguchi and Barchas (15) failed to find any change in the levels of this enzyme after treatment of mice with MK 486 or Ro 4-4602.

ACKNOWLEDGMENT

The technical assistance of Mr. G. W. Smith is gratefully acknowledged. This research was supported by a grant from the Auckland Medical Research Foundation.

REFERENCES

1. Calne, D. B., Karoum, F., Ruthven, C. R. J. & Sandler, M. (1969) The metabolism of orally administered L-Dopa in Parkinsonism. *Br. J. Pharmacol.* **37**, 57–68.
2. O'Gorman, L. P., Borud, O., Khan, I. A. & Gjessing, L. R. (1970) The metabolism of L-3,4-dihydroxyphenylalanine in man. *Clin. Chim. Acta* **29**, 111–119.
3. Sandler, M., Johnson, R. D., Ruthven, C. R. J., Reid, J. L. & Calne, D. B. (1974) Transamination is a major pathway of L-dopa metabolism following peripheral decarboxylase inhibition. *Nature (London)* **247**, 364–366.
4. Bronaugh, R. L., Wenger, G. R., Garver, D. L. & Rutledge, C. O. (1976) Effect of carbidopa on the metabolism of L-Dopa in the pigtail monkey. *Biochem. Pharmacol.* **25**, 1679–1681.
5. Fellman, J. H. & Roth, E. S. (1971) Inhibition of tyrosine aminotransferase activity by L-3, 4-dihydroxyphenylalanine. *Biochemistry* **10**, 408–414.
6. Lees, G. J. & Weiner, N. (1975) An examination of the catalytic properties of several aminotransferases in brain and liver by means of an improved radiometric assay with dinitrophenylhydrazine. *J. Neurochem.* **25**, 315–322.
7. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275.
8. Jacoby, G. A. & La Du, B. N. (1964) Studies on the specificity of tyrosine-α-ketoglutarate transaminase. *J. Biol. Chem.* **239**, 419–424.
9. Lovenberg, W., Weissbach, H. & Udenfriend, S. (1962) Aromatic L-amino acid decarboxylase. *J. Biol. Chem.* **237**, 89–93.
10. Fellman, J. H., Wada, G. H. & Roth, E. S. (1975) Metabolism and properties of 3-methoxy-4-hydroxyphenylpyruvate; a metabolite of dihydroxyphenylalanine. *Biochim. Biophys. Acta* **381**, 9–16.
11. Porter, C. C. (1973) Inhibitors of aromatic amino acid decarboxylase—their biochemistry. *Adv. Neurol.* **2**, 37–58.
12. Borri-Voltattorni, C. & Minelli, A. (1977) Interaction of L-α-methyl-α-hydrazino-3,4-dihydroxyphenyl propionic acid with Dopa decarboxylase from pig kidney. *FEBS Lett.* **75**, 277–280.
13. Fellman, J. H., Roth, E. S. & Fujita, T. S. (1976) Decarboxylation to tyramine is not a major route of tyrosine metabolism in mammals. *Arch. Biochem. Biophys.* **174**, 562–567.
14. David, J-C., Dairman, W. & Udenfriend, S. (1974) On the importance of decarboxylation in the metabolism of phenylalanine, tyrosine, and tryptophan. *Arch. Biochem. Biophys.* **160**, 561–568.
15. Deguchi, T. & Barchas, J. (1971) Induction of hepatic tyrosine aminotransferase by indole amines. *J. Biol. Chem.* **246**, 7217–7222.