Tryptophan Derivatives as Inhibitors of Tyrosine Hydroxylase *in Vivo* and *in Vitro*

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SUMMARY

Certain typtophan analogs were found to be potent inhibitors of tyrosine hydroxylase and to act by a mechanism that is not competitive with substrate. The most active compounds in these studies were those with a hydroxyl group at the 5 position on the indole ring. The most potent inhibitor was α-methyl-5-hydroxytryptophan. Amines or acids resulting from metabolism of the parent compounds were found to be inactive. Tyrosine hydroxylase activity was inhibited *in vivo* after a 50 mg/kg dose of α-methyl-5-hydroxytryptophan; a single dose of 200 mg/kg inhibited the enzyme up to 48 hr. Administration of this compound resulted in depletion of tissue stores of catecholamines as well as in sedation.

INTRODUCTION

The inhibition of tyrosine hydroxylase, the rate-limiting reaction in norepinephrine biosynthesis, results in the depletion of catecholamine stores in heart, brain, and spleen (1–3). Compounds which inhibit this enzyme may produce a "chemical sympathectomy" and are therefore useful tools for study of the sympathetic nervous system and brain (4–11). They may also serve as therapeutic agents (12). The present report concerns the *in vitro* and *in vivo* actions of several tryptophan derivatives which are potent inhibitors of tyrosine hydroxylase.[3]

METHODS

We wish to thank Dr. R. V. Heinzelman of the Upjohn Co. for α-methyl-5-hydroxy-DL-tryptophan, α-methyltryptamine, and α-methyl-5-hydroxytryptamine. We also wish to thank Dr. J. W. Daly (National Institute of Arthritis and Metabolic Diseases) for his kindness in supplying other tryptophan derivatives used in these studies. 2-Amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine ($DMPH_4$) was obtained from Aldrich Chemical Co. L-Tyrosine-3,5-^{3}H (5600 μC/μmole) was obtained from New England Nuclear Corp. and was purified by passage over Dowex 50, H^+ form, prior to use. Tyrosine hydroxylase was prepared from beef adrenal medulla by a modification of the procedure of Nagatsu *et al.* (4). The enzyme from guinea pig heart press juice was prepared as previously described (7).

With the adrenal preparation tyrosine hydroxylase activity was assayed by measuring the formation of tritiated water from tyrosine-3,5-^{3}H (13). A modification of this assay was used for the guinea pig enzyme (7). The *in vivo* inhibition of tyrosine hydroxylase was estimated as described in the earlier report (7). Compounds were dissolved in 0.1 N HCl, buffered to pH 6.0–6.5, and injected by the intraperitoneal route into guinea pigs (200–

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TABLE 1
The effect of a series of indole derivatives on tyrosine hydroxylase activity

In the experiments with purified bovine adrenal tyrosine hydroxylase the incubation mixture contained L-tyrosine-3,5-^{3}H, 150,000–200,000 cpm; tyrosine, 0.10 μmole; mercaptoethanol, 50 μmoles; $DMPH_4$, 0.5 μmole; phosphate buffer pH 6.4, 50 μmoles; enzyme, 2 mg; and water to a final volume of 0.5 ml.

When guinea pig heart press juice was used as the enzyme source the incubation mixture contained L-tyrosine-3,5-^{3}H, 300,000 cpm; mercaptoethanol, 10 μmoles; $DMPH_4$, 0.5 μmole; phosphate buffer, pH 6.4, 10 μmoles; andenzyme, 0.10 ml. The endogenous tyrosine that was found in the heart press juice served as substrate along with the tracer amount of radioactive tyrosine. The final volume of each sample was adjusted to 0.125 ml so that the concentration of the enzyme was diluted only about 20–25% and the final concentration of substrate in each sample was about 10^{-5} M.

With both preparations inhibitors were added at final concentrations of 10^{-4} M and incubation was for 15 min in air at 37°.

Compound	Inhibition (%)	
	Purified tyrosine hydroxylase	Heart press juice
α-Methyl-DL-tryptophan	—	47
α-Methyl-5-hydroxy-DL-tryptophan	76	96
α-Methyltryptamine	0	15
α-Methyl-5-hydroxytryptamine	0	0
5-Hydroxyindoleacetic acid	0	0
Indoleacetic acid	0	0

400 g) which had been fasted overnight. The animals were killed after a suitable interval and the tyrosine hydroxylase activity was measured in heart press juice (7). Details are given in Table 1. Catecholamines in various tissues were assayed by the trihydroxyindole method as described by Crout (14).

RESULTS

Structure–Activity Relationships

A number of indoles were tested for inhibitory action on two preparations of tyrosine hydroxylase, one which was purified from bovine adrenal medulla and the other a crude guinea pig heart press juice (Table 1). The greater inhibition observed in the heart enzyme with α-methyl-5-hydroxytryptophan is merely a reflection of the lower amount of tyrosine used as substrate. The amino acids inhibited tyrosine hydroxylase considerably in both preparations (see also Table 2). However, neither the acids nor amines which result from deamination or decarboxylation of the parent amino acids were active. A 5-min preincubation of inhibitors with enzyme, in the absence of tyrosine and cofactor, did not alter the degree of inhibition.

TABLE 2
Effects of various tryptophan analogs on tyrosine hydroxylase

Compounds were added to the standard incubation mixture at a concentration of 1×10^{-4} M. The purified beef adrenal enzyme was used and incubations were carried out as described in Table 1.

Compound	Inhibition (%)
L-Tryptophan	20
D-Tryptophan	0
N-Methyl-DL-tryptophan	12
α-Methyl-DL-tryptophan	17
5-Hydroxy-DL-tryptophan	30
5-Hydroxy-L-tryptophan	47
6-Hydroxy-DL-tryptophan	20
7-Hydroxy-DL-tryptophan	6
5-Hydroxy-α-methyl-DL-tryptophan	83

Since the substituted indole amino acids exhibited inhibitory activity, more of these compounds were examined. As shown in Table 2 the addition of a hydroxyl group at the 5 position confers potent inhibitory activity. The subsequent addition of an α-methyl group further enhances the activity. However, addition of a hydroxyl group at the 6 or 7 position has little effect on activity. Unsubstituted tryptophan, as well as the *N*-methyl and α-methyl derivatives of tryptophan, are relatively weak inhibitors.

Mechanism of Inhibition

The nature of the inhibition by the two most potent tryptophan analogs was

investigated further. In the double reciprocal plots shown in Fig. 1 the substrate was tyrosine. It may be seen that both 5-hydroxytryptophan and α-methyl-5-hydroxytryptophan exhibited characteristics

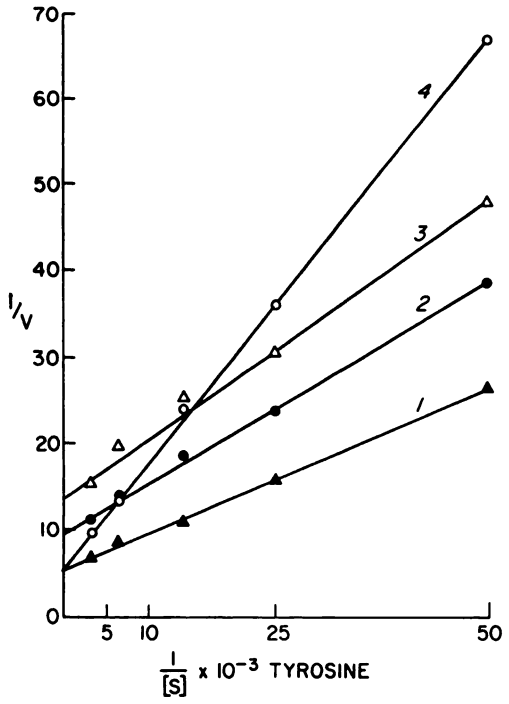

FIG. 1. *Double reciprocal plots of tyrosine concentration vs rate of tyrosine hydroxylation, with and without tryptophan analogs and α-methyltyrosine*

The compounds used were (curve *1*) tyrosine alone; and tyrosine plus (curve *2*) 5-hydroxytryptophan 1×10^{-4} M; (curve *3*) α-methyl-5-hydroxy-DL-tryptophan 2×10^{-5} M; (curve *4*) α-methyl-L-tyrosine 2×10^{-5} M. Activity was measured with the purified adrenal enzyme under the conditions shown in Table 1, at a $DMPH_4$ concentration of 1×10^{-3} M. Tyrosine was varied between 3×10^{-4} M and 2×10^{-5} M.

of uncompetitive inhibition while the inhibition by α-methyltyrosine, which was included for comparison, was competitive with the substrate, as had been shown previously (4).

Double reciprocal plots of velocity versus reduced pteridine cosubstrate are shown in Fig. 2. α-Methyl-5-hydroxytryptophan exhibited both competitive and noncompetitive inhibition depending on the concentration of cosubstrate used. At concentrations of reduced pteridine, 1×10^{-3} M and higher, competitive inhibition was seen; at concentrations lower than 1×10^{-3} M noncompetitive inhibition was observed. By contrast, inhibition by α-methyltyrosine was uncompetitive at all concentrations. The latter had already been shown in earlier studies (4).

Studies in Vivo

Tryptophan analogs which inhibited tyrosine hydroxylase *in vitro* were also effective *in vivo*. Figure 3 illustrates the relationship of the dose of α-methyl-5-hydroxy-DL-tryptophan to cardiac tyrosine hydroxylase activity when the drug was administered by the intraperitoneal route and enzyme activity was measured *in vitro* after 2 hr. Administration of 50 mg/kg of the α-methylamino acid resulted in about a 50% inhibition of tyrosine hydroxylase, the inhibition increasing with increasing dose of the drug.

The duration of tyrosine hydroxylase inhibition was investigated by killing guinea pigs at varying intervals after the administration of a single dose of α-methyl-5-hydroxy-DL-tryptophan (200 mg/kg). The results shown in Fig. 4 indicate that significant inhibition persists for 48 hr.

Although no detailed pharmacologic studies were undertaken, it was noted that guinea pigs treated with α-methyl-5-hydroxytryptophan, like those treated with α-methyltyrosine, were sedated as shown by reduced motor activity and a diminished resistance to handling. These same effects were observed in rats.

Some studies were also carried out with 5-hydroxytryptophan, which is both a serotonin precursor and inhibitor of tyrosine hydroxylase. Enzyme activity in guinea pig heart was inhibited about 50% 2 hr after the injection of 300 mg/kg of 5-hydroxy-DL-tryptophan.

Single and repeated administration of α-methyl-5-hydroxy-DL-tryptophan resulted in reduction of the norepinephrine stores in tissues comparable to those produced by α-methyltyrosine. The effects of single doses of these two inhibitors on norepinephrine levels in brain and heart are shown in Table 3. More recently Johnson and

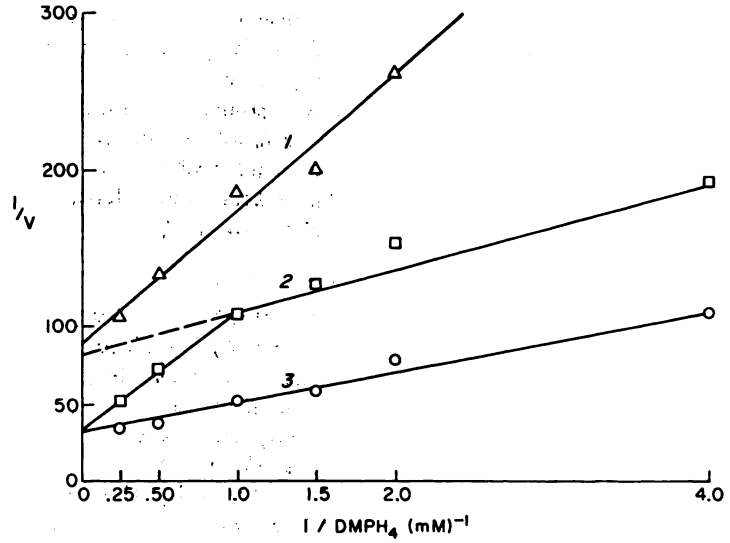

FIG. 2. *Double reciprocal plots of $DMPH_4$ concentration vs rate of tyrosine hydroxylation with α-methyl-5-hydroxytryptophan and α-methyltyrosine*

Curve *1*, α-Methyltyrosine 2×10^{-5} M; curve *2*, α-methyl-5-hydroxytryptophan 2×10^{-5} M; curve *3*, tyrosine alone. Activity was measured at a tyrosine concentration of 2×10^{-4} M, and $DMPH_4$ was varied between 4×10^{-3} M and 2.5×10^{-4} M. The enzyme system was otherwise the same as in Fig. 1.

Freyburger[4] showed that in rats a dosage regime of α-methyl-5-hydroxytryptophan, comparable to that used in the guinea pig in these studies, depletes heart norepinephrine to levels less than 10% of controls but lowers brain levels by about 50%.

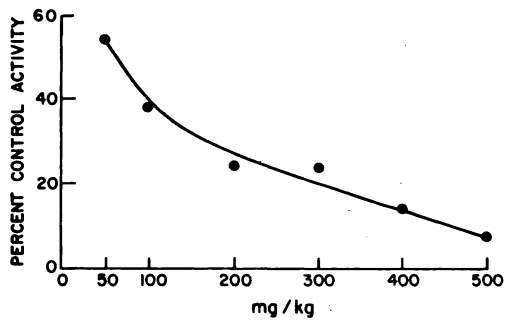

FIG. 3. *Activity of guinea pig heart tyrosine hydroxylase after administration of α-methyl-5-hydroxy-DL-tryptophan in vivo*

The animals were killed 2 hr after the injection of the compound. Tyrosine hydroxylase activity was measured in heart press juice as described under Methods. Each point represents the mean of 4 to 10 animals.

[4] G. A. Johnson and W. F. Freyburger, Upjohn Co., Kalamazoo, Michigan, personal communication.

DISCUSSION

Inhibition of the rate-limiting step of norepinephrine biosynthesis has been shown to result in a marked reduction of catecholamine stores (2, 3). α-Methyltyrosine which inhibits tyrosine hydroxylase by competing with the substrate (4), is a valuable pharmacologic and biochemical tool and is effective in treatment of pheochromocytoma (12). Although it is a potent inhibitor of the enzyme *in vivo* it is difficult to administer because of poor solubility. Furthermore, α-methyltyrosine is slowly hydroxylated to a catechol (5) and to α-methyl-norepinephrine, a false transmitter (15, 16). This metabolite may mask some of the *in vivo* pharmacologic effects of the tyrosine hydroxylase inhibition.

α-Methyl-5-hydroxytryptophan also inhibits tyrosine hydroxylase effectively *in vivo* and *in vitro*. However, it is apparently 15–20 times more soluble in water than α-methyltyrosine,[4] which makes it much easier to administer experimentally. Furthermore it is not metabolized to catecholamine derivatives. It should be noted that in the *in vivo* studies the racemic

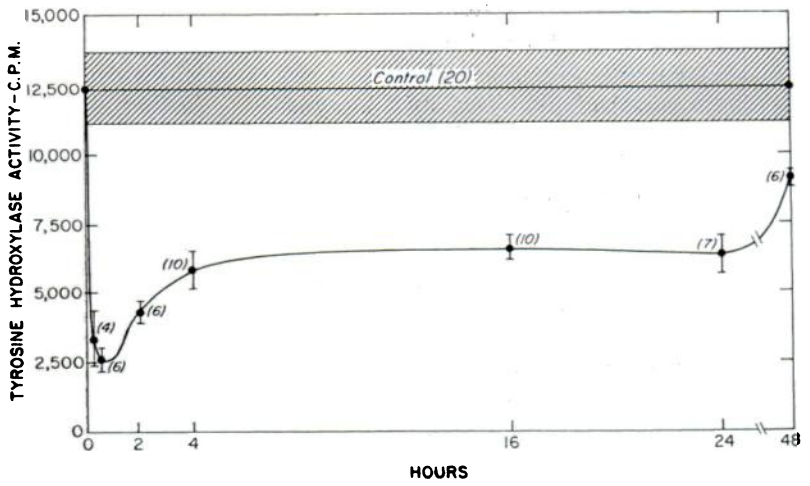

FIG. 4. *Guinea pig heart tyrosine hydroxylase activity after administration of 200 mg/kg of α-methyl-5-hydroxy-DL-tryptophan*

The animals were killed at the intervals specified, and enzyme activity of the heart press juice was measured as described under Methods. The vertical line at each point represents the standard error of the mean of the number of animals shown in parentheses.

TABLE 3

Effects of α-methyl-5-hydroxytryptophan and α-methyltyrosine on tissue levels of norepinephrine

The guinea pigs were injected 3 times a day at 4-hr intervals with 200 mg/kg α-methyltyrosine or 200 mg/kg α-methyl-5-hydroxytryptophan. The rats were given a single intraperitoneal injection of the same compounds at the same dosage and killed 6 hr later. Controls were injected with corresponding volumes of the solvent diluents. The values are presented ± standard error with the number of animals shown in parentheses.

Organ	Norepinephrine (μg/g tissue)		
	Controls	α-Methyl-5-hydroxytryptophan	α-Methyltyrosine
Guinea pig hearts	1.79 ± 0.05 (5)	0.75 ± 0.10 (5)	0.57 ± 0.09 (5)
Rat brain stem	0.53 ± 0.03 (3)	0.24 ± 0.04 (3)	0.34 ± 0.03 (3)

form of the indole derivative was compared to the L-form of α-methyltyrosine. Most probably all the activity resides in the L-form of the indole too.

α-Methyl-5-hydroxytryptophan is also a potent inhibitor of aromatic L-amino acid decarboxylase (17). However, the decrease in DOPA decarboxylation by α-methyl-5-hydroxytryptophan probably is not an important factor in reducing the tissue stores of amines since other potent DOPA decarboxylase inhibitors have been shown to have little effect on catecholamine stores as a direct result of the enzyme inhibition (15, 18, 19, 20). More recently Johnson and Freyburger[4] have shown that trace amounts of α-methyl-5-hydroxytryptamine are formed *in vivo* from α-methyl-5-hydroxytryptophan. The amine is a potent norepinephrine-releasing agent and may augment the norepinephrine-depleting action.

The structural similarities of tyrosine and 5-hydroxytryptophan and the marked inhibitory activity of the α-methyl derivatives in both cases would suggest that the indole amino acids inhibit by competing with the substrate tyrosine. It was surprising to find that the tryptophan derivatives do not compete with either tyrosine or

reduced pteridine (at physiologic levels). The exact mechanism of inhibition by α-methyl-5-hydroxytryptophan remains to be determined.[5] The reversal of inhibition by a high concentration of reduced cosubstrate may have little practical significance because of the very low concentration of pteridine cofactor in tissues (21). Because the inhibition by α-methyl-5-hydroxytryptophan is independent of substrate or pteridine, its actions should be unaffected by dietary variations.

McGeer and McGeer (9) noted a slight inhibition of tyrosine hydroxylase by 5-hydroxy-DL-tryptophan. However, these workers did not obtain a full measure of the potency of this amino acid because of the limitation of their testing procedure.[6]

In comparison with α-methyltyrosine, α-methyl-5-hydroxytryptophan decreases norepinephrine levels more markedly in heart than it does in brain. This fact may reflect either poor penetration of the compound into the brain or a lower potency toward the brain enzyme. It is also likely that some of the patterns of norepinephrine depletion *in vivo* reflect differences in decarboxylation to α-methyl-5-hydroxytryptamine.

A new insight into catecholamine and serotonin interrelationships is suggested by the fact that 5-hydroxytryptophan inhibits tyrosine hydroxylase and thereby norepinephrine synthesis. It is of further interest that this compound and its α-methyl derivative have little effect on tryptophan hydroxylation (22). It is conceivable that by depressing tyrosine hydroxylation, the serotonin precursor may participate in the regulation of norepinephrine synthesis. However, there is as yet no evidence for the accumulation of 5-hydroxytryptophan in tissues. It is interesting that norepinephrine inhibits tryptophan hydroxylation (23) and may therefore participate in the regulation of serotonin biosynthesis. Further studies on the interrelationships of catechol and serotonin biosynthetic mechanisms are warranted.

[5] Recently O. Nikodijevic and P. Zaltsman-Nirenberg, in this laboratory, have demonstrated that α-methyl-5-hydroxytryptophan is not a substrate of tyrosine hydroxylase.

[6] A communication by McGeer, McGeer and Peters [*Life Sci.* **6**, 2221 (1967)] concerning the inhibition of tyrosine hydroxylase by 5-halogenated tryptophan appeared just as this manuscript was being prepared for submission.

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