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Inhibition of Tryptophan Pyrrolase of Rats by Phenols, Tryptophan Analogs and Hydrazine Derivatives[1]

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Studies on the inhibition of tryptophan pyrrolase by eleven phenols, six tryptophan analogs and seven hydrazine derivatives were carried out. Serotonin, 5-hydroxytryptophan, epinephrine, norepinephrine and dopamine were found to inhibit the enzyme significantly. On the other hand, phenylhydrazine was the most potent inhibitor among the compounds tested. However, hydrazine hydrochloride, the parent compound of phenylhydrazine, was not inhibitory.

Since tryptophan pyrrolase (TP) was found as an inducible enzyme in rats by Knox and Mehler,[3] an amounts of reports regarding the effects of various substances on this enzyme have been appeared.

Knox and Auerbach[4] demonstrated the induction of TP by adrenocortical hormones. Schor and Frieden[5] indicated that treatment with insulin and alloxan could induce this enzyme in adrenalectomized rats. Frieden, *et al.*[6] showed the inhibition of TP by the addition of epinephrine or serotonin *in vitro* experiments.

As reported in the preceding paper,[7] two monoamine oxidase inhibitors which were associated with phenylalkylhydrazine, JB-516 and phenelzine, were found to inhibit TP significantly. Recent investigations by Cho-Chung and Pitot showed the feedback control of TP by NADPH *in vitro*[8] and *in vivo*[9] experiments.

The present study was undertaken to determine the inhibitory effects of phenols other than epinephrine, tryptophan analogs which have similar chemical structures to that of serotonin and hydrazine derivatives on the enzyme.

Experimental

Animals——Male rats of Wistar strain weighing 200—250 g were employed throughout the present study.

Materials——TP was induced by the injection of L-tryptophan in a dose of 100 mg/100 g *i.p.* Four hours later, animals were sacrificed by decapitation and the livers were removed, blotted and homogenized in a Potter-Elvehjem type homogenizer with Teflon pestle in 7 volumes of 0.14M KCl containing 0.0025N NaOH solution[10] to serve as a source of the enzyme.

Assay Method——The enzyme activity was assayed by a modification[10] of the method of Knox and Mehler.[3]

1) Presented to the 23rd Annual Meeting of the Pharmaceutical Society of Japan, Sendai, October 1966 and to the 17th Regional Meeting of the North Area of the Japanese Pharmacological Society, Hirosaki, October 1966.
2) Location: *Izumi-cho, Narashino, Chiba.*
3) W.E. Knox and A.H. Mehler, *J. Biol. Chem.*, **187**, 419 (1950).
4) W.E. Knox and V.H. Auerbach, *J. Biol. Chem.*, **214**, 307 (1955).
5) J.M. Schor and E. Frieden, *J. Biol. Chem.*, **233**, 612 (1958).
6) E. Frieden, G.W. Westmark, and J.M. Schor, *Arch. Biochem. Biophys.*, **92**, 176 (1961).
7) T. Satoh and T. Iwamoto, *Jap. J. Pharmacol.*, **18**, 89 (1968).
8) Y.S. Cho-Chung and H.C. Pitot, *J. Biol. Chem.*, **242**, 1192 (1967).
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10) P. Feigelson and O. Greengard, *J. Biol. Chem.*, **236**, 153 (1961).

When all experiments were carried out, blanks with each compound were subtracted from the enzyme activities obtained with samples.

In all tables in this paper, the data have been expressed as per cent activity of control in the presence of indicated concentrations of the compounds.

Concentrations of Compounds——Various compounds were used in aqueous solutiosn of 1×10^{-3}, 1×10^{-4} and 1×10^{-5}M as final concentrations in incubation mixture.

All experiments were performed in duplicate and results were shown as mean values of three experiments.

Results

Effects of Phenols on TP

The results on phenols and catechols were illustrated in Table I. It is postulated that the inhibitory effects of the compounds in this series on TP may be potentiated by hydroxylation of benzene ring, because the best inhibition of TP was obtained with catechols rather than phenols.

Table I. Inhibition of Tryptophan Pyrrolase by Phenols

Compound	Concentration (M)		
	1×10^{-3}	1×10^{-4}	1×10^{-5}
None	100	100	100
Phenylalanine	89	95	93
Tyrosine	48	94	100
DOPA	37	43	85
Dopamine	36	50	89
α-Methyl DOPA	24	58	94
3,4-Dihydroxymandelic acid	56	87	97
Norepinephrine	18	47	87
Epinephrine	48	72	95
Phenol	42	78	100
Salicylate	36	67	81
Benzoate	71	82	96

All compounds used in this study were added at the beginning of incubation.
Figures in tables throughout the present paper indicate TP activity as percentage of that of control experiment.

Epinephrine and norepinephrine, as shown by Frieden, *et al.*,[6] demonstrated the potent inhibition of TP. Benzoate also showed the inhibition of the enzyme and significant increase in inhibition of TP was recognized by addition of salicylate.

Effects of Tryptophan Analogs

Six compounds associated with tryptophan were examined, and as illustrated in Table II, the most inhibitory effect of 5-hydroxytryptophan on TP was recognized. Serotonin,

Table II. Inhibition of Trypotphan Pyrrolase by Tryptophan Analogs

Compound	Concentration (M)		
	1×10^{-3}	1×10^{-4}	1×10^{-5}
None	100	100	100
5-Hydroxytryptophan	46	84	100
Serotonin	68	95	100
5-Hydroxyindole acetic acid	82	100	100
Bufotenine	56	91	100
Tryptamine	75	100	100
Indole	65	100	100

tryptamine and 5-hydroxyindole acetic acid which is known as major metabolite of serotonin in urine showed less inhibitory effects, and bufotenine, dimethylaminoserotonin, was found to be moderately effective.

Indole also showed inhibitory effect as well as serotonin. Inhibitory effects of these compounds were observed only in the presence of higher concentration of inhibitor (1×10^{-3}M).

Effects of Hydrazine Derivatives on TP

Effects of hydrazine and six related compounds on TP were examined and results were summarized in Table III, in which hydrazine, iproniazid, benzylamine and INH had no effects on the enzyme, however, urea and thiourea showed moderate inhibition on TP, and the most potent inhibition of the enzyme was observed when phenylhydrazine in the concentration of 1×10^{-3}M was added in incubation medium.

Discussion

With regard to the inhibition of TP, one possibility is postulated that TP is inhibited competitively by the substrate analogs as shown by 5-methyltryptophan and tryptazan[11] and 3-hydroxyanthranilate.[12] A second one is that aromatic amines or amino acids seem to act the site other than the substrate one, resulting some change of the protein conformation which modifies its activity.

As shown in Table I, certain phenols inhibited TP. It is likely that inhibitory effects of these compounds were arosed by formation of complex between phenols and heme iron, since interaction of both compounds is well documented.

On the other hand, the findings that 5-hydroxytryptophan and serotonin have inhibitory effects on TP are of importance in studying tryptophan metabolism. Although the mechanism of inhibition of TP by these compounds remains obscure, one possibility is that some sort of charge transfer complex forms between TP and indole derivatives, since these compounds contain strong electronegative group in the indole nucleus.

Furthermore, inhibitory effects of the compounds, as seen in Tables I and II, appeared to be scarecely modified by decarboxylation of the compounds.

As illustrated in Table III, inhibition of TP by the compounds in this series may attribute to hydrazino group, but not omega amino group in side chain of their aromatic structures. Since inhibition of catalase by phenylhydrazine was reported by Andrejew, *et al.*[13] and

Table III. Inhibition of Tryptophan Pyrrolase by Hydrazine Derivatives

Compound	Concentration (M)		
	1×10^{-3}	1×10^{-4}	1×10^{-5}
None	100	100	100
Urea	90	100	100
Thiourea	89	96	100
Hydrazine	91	100	100
Phenylhydrazine	0	6	30
Iproniazid	100	100	100
Benzylamine	100	100	100
Isonicotinyl hydrazid	100	100	100

11) M. Civen and W.E. Knox, *J. Biol. Chem.*, 235, 1716 (1960).
12) C. Wagner, *Biochem. Biophys. Res. Commun.*, 17, 668 (1964).

Cohen and Hochstein[14] and, in addition, the fact that this compound causes decrease in biosynthesis of erythrocyte has been well known,[15] it is assumed that the heme enzyme may be inhibited by phenylhydrazine.

In vivo effects of this compound are currently under investigation.

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14) G. Cohen and P. Hochstein, *J. Pharmac. Exp. Therap.*, **147**, 139 (1965).
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