THE EFFECT OF AROMATIC AMINO ACIDS ON THE HYDROXYLATION OF TRYPTOPHAN *

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The acute interest between disturbances in phenylalanine and tryptophan metabolism which originally led to the finding of a tryptophan hydroxylating system (THS) in liver (Freedland, et al, 1961) has led to many new findings. A recent report indicates that the low excretion of 5-hydroxyindole acetic acid and low blood 5-hydroxytryptamine (5-HT) levels after feeding excess phenylalanine and tyrosine are secondary to the increase of these amino acids in the blood (Huang, et al, 1961). It has also been shown that excessive feeding of phenylalanine causes a significant decrease of brain 5-HT in rats (Yuwiler & Louttit, 1961). In both of these reports 5-hydroxytryptophan (5-HTP) decarboxylase has been indicated as the possible site of inhibition since various phenylalanine metabolites have been shown to inhibit 5-HTP decarboxylase in vitro (Davison & Sandler, 1958). However, if this were the case one should find an accumulation of 5-HTP which has not been shown to date. Therefore, the observed inhibition may occur at an earlier step, namely the

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hydroxylation of tryptophan. This led to the present study on the effect of certain aromatic amino acids and their derivatives on the THS *in vitro*.

The THS was prepared from livers of male albino rats as previously described (Freedland, *et al*, 1961). During this time it was found that the entire THS was in the soluble fraction after 60 minutes at 135,000xg in a Spinco Model L preparative ultracentrifuge and both this and the 20,000xg preparations of the THS were used interchangeably since both preparations gave the same activity under the assay conditions. All assays contained 1.0 ml of THS in a volume of 2.5 ml, and were carried out for one hour. The hydroxylated product was determined by a modification of the 1-nitros-2-napthol reaction (Udenfriend, *et al*, 1955).

Phenylalanine and some of its metabolites such as tyrosine, phenyllactic acid and phenylpyruvic acid do inhibit the THS system quite markedly. (Table 1). Apparently the compounds containing a carboxyl group and a β-aromatic ring in conjunction with an amino, hydroxy or ketone group in the 2 position of the side chain inhibit this reaction. It also appears that if the α-carbon is optically active only the compounds with the L configuration have inhibiting properties. Further studies on the phenylalanine inhibition of the THS indicate that the inhibition is competitive. Although the reaction is carried out with a crude supernate fraction and is at least trimolecular, an attempt was made to estimate the apparent Km' for L-tryptophan and the apparent K_I' for L-phenylalanine by keeping the DPN^+, O_2,

TABLE 1

EFFECT OF PHENYLALANINE AND RELATED COMPOUNDS ON THE HYDROXYLATION OF TRYPTOPHAN*

Compound	M. x 10^{-4}	%Inhibition of THS (a)	(b)
L-phenylalanine	4	70	71
L-phenylalanine	2	45	37
DL-phenylalanine	4	42	43
DL-phenylalanine	2	25	
L-tyrosine	2.2	32	32
phenylpyruvic	4	62	45
DL-phenyllactic	4	38	36
L-phenylalanine+	2		
L-tyrosine	1.1		51
L-phenylalanine+	2		
phenylpyruvic	2		54

*The concentration of L-Tryptophan was 1.6 x 10^{-2} in all above assays.
The following compounds did not inhibit at 4x10^{-4}M: phenylacetic acid and phenylethylamine, D-phenylalanine, L-alanine, L-leucine, and NH_4^+ as $(NH_4)_2 SO_4$ or NH_4Cl.
(a) and (b) are two separate determinations on tissues from different animals.

and intrinsic factors relatively constant. The Km' ranged from 1 to 5 x 10^{-2}M for L-tryptophan with an average of 2.9 x 10^{-2}M. The K_I for L-phenylalanine ranged between 0.5 to 4.0 x 10^{-4}M with an average of 2.2 x 10^{-4}M. This latter value of 2.2 x 10^{-4}M for the K_I' of L-phenylalanine is very close to 2.0 x 10^{-4}M the Km' measured for L-phenylalanine in the phenylalanine hydroxylase reaction as determined by a modification of the method of Udenfriend & Cooper, 1952.

These observations suggested the possibility that the enzymes for the hydroxylation of L-phenylalanine and L-tryptophan were the same. This does not necessarily mean that the co-factor or other enzymes required for the hydroxylation is also identical. If the two hydroxylases are identical one should expect not only

L-phenylalanine to inhibit the hydroxylation of L-tryptophan but vice-versa: Indeed L-tryptophan does inhibit the hydroxylation of L-phenylalanine (Table II),

Table II
Inhibition by Phenylalanine

M of Phenylalanine x 10^{-4}	% inhibition of THS*	M Trpytophan x 10^{-4}	% inhibition of Phenylal.H.**
.125	6	6.25	5
.25	9	12.5	11
.50	21	25	21
1.0	32	50	35
2.0	47		
4.0	83		
8.0	94		

*L-Tryptophan concentration was 1.6 x 10^{-2}
**L-Phenylalanine concentration was 8 x 10^{-4}

although not as drastically as the reverse inhibition. Since both the Km' and K_I' are related to the affinity of the substrate for the enzyme it would be expected that L-phenylalanine would be the more effective inhibitor of the two. Since it has been shown that age, sex, and dietary regimen influence the liver phenylalanine hydroxylase activity (Freedland, et al, 1960) both this activity and the THS activity were measured at different ages in both sexes and under various dietary regimens. When the phenylalanine hydroxylase activity was plotted against the THS activity there was a high degree of correlation; r=0.962.

Thus, phenylalanine and tryptophan hydroxylase are either the same enzyme or are affected by the same factors in the same direction. This does not necessarily mean that a genetic defect in one system will cause the same defect in the second system. For example, if part of the active center of the enzyme were common to both substrates it is possible that the ability to hydroxylate phenylalanine may be lost without a complete loss

of affinity for this substrate and thus phenylalanine could still be a competitive inhibiter of the THS. Under these conditions the phenylalanine would still be able to inhibit the hydroxylation of tryptophan by competing for the active site and one would expect metabolic patterns of hydroxyindole as have been observed in phenylketonurics (Baldridge, et al, 1959). Thus, it is possible that although the primary lesion in phenylketonuria is the inability to hydroxylate phenylalanine the more critical deficiency may be for 5-HTP and 5-HT during the time of most rapid brain development.

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