Metabolism of 5-Hydroxy-D- and L-Tryptophan in Man: Synthesis of Serotonin from the D-Isomer. (26910)

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It is generally assumed that formation of 5-hydroxytryptamine (serotonin) and 5-hydroxyindoleacetic acid (5HIAA) from 5-hydroxy-DL-tryptophan (5HTP) is due only to metabolism of the L-isomer. However, the actual fate of the D-isomer has not been determined. It is important that this be ascertained since the 5HTP which is available commercially is the racemic mixture of its D- and L-isomers, and thus is the only form which has been used for pharmacologic and biochemical studies in man. It has been shown that D-5HTP is not a substrate for aromatic L-amino acid decarboxylase(1), and could not therefore serve as an immediate precursor of serotonin. However, the theoretical possibility existed that formation of 5HIAA from the D-isomer might be effected by D-amino acid oxidase *via* the intermediate, 5-hydroxyindolepyruvic acid. The resolution of 5HTP into its optical antipodes by Morris and Armstrong(2) afforded the opportunity to study this possibility.

Quite unexpectedly, it was found that infusion of D-5HTP into human subjects resulted in increased urinary excretion of not only 5HIAA, but also serotonin. In this investigation the 5-hydroxyindole metabolites of both D- and L-5HTP have been determined following infusion of each isomer separately, and the urinary serotonin formed from D-5HTP has been identified chemically.

Methods. The 2 subjects of the study were 23 years of age, a male normal volunteer and a female patient with very mild uncomplicated primary hypertension; both were hospitalized at the Clinical Center of Nat. Inst. of Health. All fruit and fruit juices were excluded from their diets.

Small amounts of the D- and L-isomers of 5HTP were made available through the generosity of Dr. Marvin D. Armstrong, Fels Research Institute, Yellow Springs, Ohio. DL-5HTP was purchased from a commercial source. After being heated in the powder form at 80°C for 45 minutes, the various forms of 5HTP were dissolved in 150 ml of 0.9% NaCl and infused intravenously at a constant rate over periods of 150 minutes in subject B.W. and 75 minutes in L.M. Urine was collected for 12 hours after beginning each infusion. Urines for control assays were collected for 12 hours following infusion of 150 ml of 0.9% NaCl and also during corresponding 12-hour periods in which there were no infusions.

Urinary L-5HTP was measured by fluorometric determination of the serotonin formed after incubation with aromatic L-amino acid decarboxylase(3). Serotonin was separated from the urine on a weakly acidic cation exchange resin and measured fluorometrically (4). Urinary 5HIAA was measured by colorimetric determination of its 1-nitroso-2-naphthol complex(5). Two-dimensional chromatography of the urine was performed as described by Jepson(6).

The purity of the D- and L-5HTP was established initially by Morris and Armstrong (2) and was confirmed in this laboratory as follows: These compounds were chromatographed in a 2 dimensional system(6), and only one Ehrlich reacting spot was present in each case. To demonstrate that it was entirely free of L-5HTP, the D-isomer was incubated with partially purified aromatic L-amino acid decarboxylase from guinea pig kidney(7). While L-5HTP was nearly quantitatively converted to serotonin by this enzyme, no serotonin was formed from the D-5HTP.

Results. Quantification of 5-hydroxyindole metabolites. The amounts of L-5HTP, serotonin and 5HIAA excreted in the 12 hours following separate infusions of D-, L- and DL-5HTP are presented in Table I, and are compared with corresponding values from control periods. Following infusion of 60 mg of D-5HTP, there was an average increment in 5HIAA excretion of 2.5 mg/12 hr. Further-

TABLE I. Excretion of 5-Hydroxyindoles Following Infusion of the Racemic Mixture (DL-) and Separate D- and L-Isomers of 5HTP.

Subject	Infusion	Urinary excretion of 5-OH-indoles		
		L-5HTP	Serotonin	5HIAA
		mg/12 hr		
B.W.	.9% NaCl	—	<.2	1.4
	None	<.5	<.2	2.3
	"	—	<.2	1.2
	D-5HTP 60 mg	<.5	1.3	4.5
	D-5HTP 60 "	—	1.5	4.3
	L-5HTP 30 "	3.2	5.2	13.5
	DL-5HTP 60 "	3.0	5.5	12.1
L.M.	.9% NaCl	—	<.2	3.4
	None	<.5	<.2	3.0
	D-5HTP 60 mg	<.5	1.8	5.4
	L-5HTP 30 "	3.3	4.8	14.4
	DL-5HTP 60 "	3.5	6.6	19.2

more, excretion of serotonin was increased from scarcely measurable amounts (<0.2 mg/12 hr) to an average of 1.57 mg/12 hr. It is apparent, therefore, that conversion to serotonin and 5HIAA represents a definite though small pathway of metabolism of D-5HTP, accounting for approximately 8% (on a molar basis) of the infused dose. No measurable L-5HTP was excreted after infusion of the D-isomer. Semiquantitative estimation of 5HTP on 2-dimensional chromatography indicated that most of the D-amino acid (70-90%) was excreted into the urine unchanged. No indole metabolites other than these mentioned were seen on chromatography of the urine following D-5HTP.

In contrast, 59-65% (on a molar basis) of L-5HTP was excreted as the sum of 5HIAA and serotonin. Of the 0.136 mM of L-5HTP infused, an average of 11% was excreted as L-5HTP, 21% as serotonin, and 42% as 5HIAA. It may also be seen that most of the serotonin and 5HIAA excreted following infusion of DL-5HTP is formed from the L-isomer.

Identification of serotonin excreted after infusion of D-5HTP. To characterize the apparent serotonin chemically, several samples of urine collected after D-5HTP infusion were carried through the column separation step of the serotonin assay(4). The eluates of these columns were pooled, and carried through a butanol extraction procedure(5) to purify the apparent serotonin further. Authentic serotonin was carried through the ion exchange and extraction steps in the same manner. The fluorescence properties of apparent serotonin were determined on a 1 ml aliquot of the final acid extract, to which 0.3 ml of 12 N HCl had been added. Both the excitation and the fluorescence spectra were found to be indistinguishable from those of authentic serotonin. The wavelengths of excitation and fluorescence maxima were 300 mμ and 540 mμ, respectively (wavelengths uncorrected).

For chromatographic characterization, an aliquot of the acid extract was adjusted to a neutral pH, evaporated under a jet of nitrogen, and desalted with ethanol: acetone (5:1). This was evaporated under a nitrogen jet to 0.2 ml, which was then applied to Whatman #1 chromatographic paper alongside a sample of authentic serotonin. An ascending chromatogram was developed with n-butanol: glacial acetic acid: H_2O (120:30:50) and dipped in Ehrlich's reagent. Ehrlich reacting spots with identical blue colors and identical Rf values (0.52) appeared at the sites of authentic and apparent serotonin.

Discussion. The metabolism of most of the infused L-5HTP to serotonin and 5HIAA was predictable from previous studies on this isomer *in vitro* and in animals(1). The appearance of appreciable quantities of serotonin in the urine after infusion of D-5HTP was certainly not expected, however, for it had not been demonstrated previously that this D-amino acid could be metabolized to serotonin. Since no serotonin was formed when D-5HTP was incubated *in vitro* with aromatic L-amino acid decarboxylase, it would not appear that serotonin is synthesized directly from the D-amino acid by action of this enzyme. Probably, formation of serotonin *in vivo* requires conversion of the D-5HTP to its L-antipode. In studying the inversion of D-phenylaminobutyric acid to its enantiomorph, duVigneaud *et al.*(8) demonstrated that the keto acid was an intermediate in such an inversion. Therefore, it is likely that inversion to L-5HTP would begin with metabolism of D-5HTP to 5-hydroxyindolepyruvic acid, a reaction catalyzed by

the flavoprotein enzyme, D-amino acid oxidase. While some 5-hydroxyindolepyruvic acid may be converted directly to 5HIAA, this keto acid intermediate also could be metabolized to L-5HTP by transamination, a reaction in which 5HTP is known to participate(9).

The only mammalian tissues known to contain D-amino acid oxidase are liver and kidney(10), and the kidney has by far the greater concentration of the enzyme. Thus, it is likely that much of the conversion of D-5HTP to its L-antipode took place in renal tissue. Since the kidney is also a rich source of L-aromatic amino acid decarboxylase, it seems likely that this organ is the chief site of the conversion of D-5HTP to serotonin. This would be compatible with the observation that D-5HTP has no pharmacologic effect on the central nervous system(1) where there is little or no D-amino acid oxidase. If further studies confirm that conversion of D-5HTP to serotonin occurs chiefly in the kidney, the use of this D-amino acid may provide an interesting approach to investigating the direct effect of serotonin on renal mechanisms.

Although constituting a relatively minor pathway, metabolism of D-5HTP to serotonin and 5HIAA should certainly be borne in mind in studies involving the D-isomer or DL-racemic 5HTP. Furthermore, the evidence that D-5HTP is converted to its L-antipode in humans probably does not represent a phenomenon specific for this amino acid. It is known that many D-amino acids may be substituted for their enantiomorphs in the diets of animals(11), and that the D-isomers of several non-physiologic amino acids may be inverted to their L-antipodes *in vivo*(8,12). All of these findings suggest that some conversion to an L-antipode may be a common pathway in the metabolism of many D-amino acids in man.

Summary. As anticipated, the metabolism of 5-hydroxy-L-tryptophan infused in humans was found to result in excretion in the urine of approximately 20% of the infused dose as serotonin and 40% as 5-hydroxyindoleacetic acid. It was quite unexpected, however, to find that conversion to serotonin and 5-hydroxyindoleacetic acid also represents a definite though small (8%) pathway for metabolism of 5-hydroxy-D-tryptophan. The significance of the latter observation is discussed and the suggestion made that serotonin synthesis from the D-amino acid probably occurs *via* formation of its antipode through the action of D-amino acid oxidase and transamination.

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