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ALTERNATIVE PATHWAYS OF 5-HYDROXINDOLE METABOLISM—I

A COMPARISON OF D-AND L-5-HYDROXYTRYPTOPHAN METABOLISM IN THE RAT

JOSEPHINE ARENDT, S. F. CONTRACTOR* and M. SANDLER†

Bernhard Baron Memorial Research Laboratories and Institute of Obstetrics and Gynaecology, Queen Charlotte's Maternity Hospital, London W.6

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Abstract—A quantitative comparison between the metabolism of the optical isomers of ^{14}C-5-hydroxytryptophan in the rat is described with and without monoamine oxidase block. Both isomers are vigorously metabolized. As large amounts of 5-hydroxytryptamine appear in the urine after D-5-hydroxytryptophan administration as after dosage with the L-isomer. The pathway of D-5-hydroxytryptophan metabolism probably leads via D-amino acid oxidation to 5-hydroxyindolepyruvic acid which undergoes transamination to L-5-hydroxytryptophan. This compound is then converted to the amine by direct renal decarboxylation.

IT IS ONLY recently that the optically active isomers of DL-5-hydroxytryptophan (5HTP) have separately become commercially available.‡ Most of the large number of experimental studies in the literature which have employed the amino acid as a research tool[1–5] have therefore made use of the DL-racemic mixture, tacitly assuming the D-optically active isomer to be metabolically inert. Indeed, 5HTP decarboxylase, the mammalian enzyme bringing about the direct conversion of 5HTP to the biologically active 5-hydroxytryptamine (5HT) is known to be L-specific;[6] Freter *et al*,[6] one of the few groups to consider that D-5HTP might undergo biological degradation, found that its administration did not increase brain 5HT concentration and presumed from this evidence that it was unchanged by the animal organism.

Apart from some inconclusive data obtained during the injection of ^{14}C-DL-5HTP in a patient with the carcinoid syndrome,[7] there appears to be only one report in the literature which presents evidence counter to this view. Oates and Sjoerdsma[8] found that intravenous injection of D-5HTP in man apparently resulted in an 8 per cent conversion to urinary 5HT and 5-hydroxyindoleacetic acid (5HIAA). Although their limited series of experiments was suggestive, it was not conclusive.

Whilst the administration of isotopically labelled optical isomers of 5HTP was obviously desirable to simulate physiological dosage and to establish the origin and simplify the isolation of true metabolites, the cost of their preparation by classical

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* Present address: Department of Obstetrics and Gynaecology, Charing Cross Hospital, London W.C.2.

† Address requests for reprints to Dr. M. Sandler, Queen Charlotte's Maternity Hospital, Goldhawk Road, London W.6.

‡ California Corp. for Biochemical Research: 3625 Medford Street, Los Angeles 63, California, U.S.A.

chemical separation procedures[6, 9, 10] would have been prohibitive. However, when a small-scale chromatographic separation procedure for optically-active isomers of tryptophan and some of its hydroxylated metabolites was recently achieved in this laboratory,[11] we were presented with the opportunity of reinvestigating this problem.

EXPERIMENTAL

Separate D- and L-isomers of ^{14}C-5HTP (sp. act. of each, 12·9 μc/mg) were prepared from ^{14}C-DL-5HTP (Radiochemical Centre, Amersham) by the method of Contractor and Wragg.[11]

Four groups of three black hooded male rats, strain PVG/C (A.R.C. Unit, Compton, Berks.), weighing approx. 200 g were used. Two groups were treated with the monoamine oxidase (MAO) inhibitor pargyline (50 mg/kg), (kindly provided by Abbott Laboratories), by intraperitoneal injection. After 5 hr, one pargyline and one untreated group were injected i.p. with 0·75 μc ^{14}C-D-5HTP whilst a similar dose of ^{14}C-L-5HTP was administered to the other pargyline and untreated group. Single animals were also given 0·75 μc of ^{14}C-D or ^{14}C-L-5HTP after treatment with 100 mg/kg pargyline 5 hr previously. Individual 24 hr urine samples were then obtained using a metabolic cage (Aerosol Products (Colchester) Ltd.), during which time water was allowed *ad libitum*, but no food.

DL-5HTP, 5HT and 5-hydroxyindoleacetic acid (5HIAA) (1 mg each) were added as carriers to the urine collection vessels, together with 6N-HCl (1 ml).

Total excreted radioactivity was measured by counting 0·1 ml portions of filtered urine on a Packard "Tricarb" Liquid Scintillation Spectrometer in polythene vials containing the following mixture: 0·1 ml urine; 4 ml 97·5% (v/v) ethanol; 16 ml liquid scintillator (600 mg 1,4-bis-2-(4-methyl-5-phenyloxazoyl)-benzene; 10 mg 2,5-diphenyloxazole; 2l redistilled toluene). All samples were counted to a standard error of ± 1 per cent; counts were corrected for quenching by the addition of internal standard and recounting.

After portions had been taken for total activity measurements, urine samples were freeze-dried, prior to further manipulation.

Freeze-dried specimens were reconstituted in a small volume of water. Portions equivalent to 2 per cent of the 24 hr urine vol. were streaked on to 4 cm wide strips of Whatman No. 1 filter paper which were run as descending chromatograms in a butanol: acetic acid: water (12–3–5) solvent system. Chromatograms were then scanned with an Atomic Accessories 4π-Scanogram. Areas of radioactive peaks on each scanogram tracing were measured planimetrically and expressed as percentages of injected radioactivity. A linear relationship had previously been established between area and counts applied to the chromatogram over the range of values studied.

5-Hydroxyindoles were located on chromatograms either by Ehrlich's reagent or by their specific pink fluorescence in ultraviolet light (254 mμ) when sprayed with dilute hydrochloric acid.[12] By using methanolic (0·1N in 99% (v/v) methanol) instead of aqueous hydrochloric acid, further spreading of spots was minimized and papers could be dried rapidly. The identities of radioactive peaks in some of the samples was further confirmed by two-dimensional chromatography[13] followed by radioautography on Kodirex X-ray film (Kodak, Ltd.)

RESULTS

On all tracings of urine samples, there were two distinct peaks whose radioactivity coincided with the location of carrier 5HT (R_f = 0·65) and 5HIAA (R_f = 0·79) respectively. Two other diffuse peaks were present; one corresponded with carrier 5HTP (R_f = 0·30) whilst the other (R_f = 0·11), which could not be identified with certainty, was presumed from two dimensional chromatography, to consist of conjugates of 5HTP or its metabolites. The position of 5HT was displaced by urea; when this area of the chromatogram was eluted with water, freeze-dried and run again in butanol: acetic acid: water side by side with an authentic 5HT marker, an R_f value of 0·48 was obtained.

Total urine radioactivity for each group of three animals expressed as a percentage of injected dose, together with the proportion of each peak making up this value, are shown in Table 1. Findings in animals pretreated with 100 mg/kg pargyline are presented in Table 2.

TABLE 1. MEAN URINARY RECOVERY VALUES OF LABELLED 5-HYDROXYINDOLES

	^{14}C-D-5HTP		^{14}C-L-5HTP	
	Without pargyline	With pargyline	Without pargyline	With pargyline
"Conjugates"	4·4	9·6	14·0	19·3
5HTP	42·6	28·2	20·6	12·4
5HT	25·0	26·5	25·9	23·0
5HIAA	6·0	3·2	32·1	13·5
Recovery of injected counts	78·0	67·5	92·6	68·2
Injected 5HTP metabolized	35·4	39·3	72·0	55·8

Expressed as percentage of injected activity, during a 24-hr period following i.p. injection of 0·75 μc ^{14}C-D- or ^{14}C-L-5HTP in groups of three rats, with and without pargyline (50 mg/kg) pretreatment.

TABLE 2. URINARY RECOVERY VALUES OF LABELLED 5-HYDROXYINDOLES

	^{14}C-D-5HTP	^{14}C-L-5HTP
"Conjugates"	3·3	22·5
5HTP	9·1	2·6
5HT	5·3	12·7
5HIAA	0·6	4·4
Recovery of injected counts	18·3	42·2
Injected 5HTP metabolized	9·2	39·6

Expressed as percentage of injected activity, during a 24 hr period following i.p. injection of 0·75 μc ^{14}C-D- or ^{14}C-L-5HTP in rats pretreated with 100 mg/kg pargyline.

None of the rats appeared to suffer any obvious clinical side-effects from the administered drugs.

DISCUSSION

In a dosage of 50 mg/kg the MAO inhibitor pargyline[14] appeared to cut down the excretion of total activity (Table 1), an effect which was exaggerated when 100 mg/kg was injected (Table 2). This action, apparently unconnected with its MAO inhibitory effect, probably arises from a decrease in glomerular filtration rate.[15, 16] It was an unforeseen complication during the present series of experiments. Further work is therefore in progress to observe the effect of MAO inhibition on 5HTP metabolism using MAO inhibitory drugs with an *in vivo* action free from renal side-effects.

After the injection of L-5HTP, the pattern of urinary metabolites was, in general, similar to the findings of Oates and Sjoerdsma.[8] About a quarter was excreted into the urine as 5HT, presumably as a result of direct renal decarboxylation.[2, 17–21] MAO block with 50 mg/kg pargyline appeared to affect 5HT output little, although to what extent any increase which might have been theoretically predicted was masked by the renal action of pargyline is unknown. This renal effect is presumably responsible for the observed drop in output of unchanged 5HTP in pargyline-treated groups. The decrease in 5HIAA and increase in conjugates in urine excreted by blocked animals was in agreement with the findings of others.[22, 23]

The preliminary observations of Oates and Sjoerdsma[8] concerning the *in vivo* breakdown of D-5HTP are fully confirmed in the present work. The degree of metabolic change in the rat (5HT plus 5HIAA = 31 per cent) was even more striking than that observed by these workers in man (5HT plus 5HIAA = 8 per cent) where 70–90 per cent of D-amino acid was excreted unchanged. Their D-5HTP dosage was given intravenously however, whereas the intraperitoneal route was used for the present work. Airaksinen[23] has indicated that speedier access of 5HT to the circulation results in a greater degree of conjugation although Oates and Sjoerdsma[8] failed to detect spots other than 5HTP, 5HT and 5HIAA on their chromatograms.

A relatively small proportion of D-5HTP was converted to 5HIAA or 5HT conjugates. After monoamine oxidase block these compounds showed a similar inverse change to that observed after L-5HTP administration to the blocked animal. The most dramatic finding was that just as much 5HT is excreted after D- than after L-5HTP injection, despite the fact that only 35·4 per cent of the isomer was metabolized compared with 72·0 per cent of the L-form. By analogy with the findings of Oates and Sjoerdsma,[8] the relatively large amounts of 5HTP excreted after D-5HTP injection are likely to have consisted almost wholly of unchanged D-isomer, without measurable contamination by the L-form.

5HTP shares many metabolic pathways with 3,4-dihydroxyphenylalanine (DOPA). It has been known for many years that D-DOPA undergoes biological degradation in the mammalian organism.[24] Sourkes and his colleagues[25, 26] have studied these changes in detail and agree with the view of Pellerin and D'Iorio[27] that, in common with many other D-amino acids,[28] D-DOPA is initially metabolized by D-amino acid oxidase.

Oates and Sjoerdsma[8] have interpreted the observed yield of urinary 5HT following D-5HTP administration in a similar manner. Following oxidative deamination by D-amino acid oxidase, the resulting 5-hydroxyindolepyruvic acid undergoes transamination to L-5HTP which is then decarboxylated to 5HTP by the highly specific 5HTP decarboxylase.[6] The present findings lead us to agree with this view.

In the rat, most D-amino acid oxidase is located in the kidney.[29] Smaller amounts are present in other sites including the brain[30, 31] although the absence of an increase in brain 5HT concentration after D-5HTP injection[6] appears to indicate that its *in vivo* metabolic role in this location is limited. The finding that much unchanged

D-5HTP is excreted after its injection and the fact that the amino acid is a relatively poor substrate for the D-amino acid[6] suggest that this enzymatic step is rate limiting. It may be that D-DOPA is a better substrate of D-amino acid oxidase than D-5HTP, as D-DOPA administration leads to a higher urinary excretion of dopamine than L-DOPA;[25] present results indicate that the yield of 5HT from D- or L-5HTP is similar. Whilst 5HTP transaminase activity has only so far been detected in liver[32, 33] and brain,[34, 35] it seems likely that high concentrations are present in the kidney; had transamination occurred in extra-renal sites during the present series of experiments, higher urinary concentrations of 5HIAA and lower levels of 5HT might have been predicted[21] together with a higher output of conjugates. In experiments involving ^{15}N-glutamic acid loading of the whole animal, Arendt *et al.*,[36] have obtained direct evidence of such transamination by isolating ^{15}N-5HT following D-5HTP administration. Thus evidence continues to accumulate to suggest that much of the early work on 5HTP metabolism, which presumed that the L-isomer only is metabolized, must undergo re-evaluation.

Whether minor routes of D-5HTP metabolism will be found to supplement the major pathway proposed by Oates and Sjoerdsma[8] must await future experiment. It is possible that a proportion of the 5-hydroxyindolepyruvic acid formed by D-amino acid oxidation is oxidatively decarboxylated to 5HIAA. Whilst this route might account for the traces of 5HIAA formed from D- (or L-)5HTP after pretreatment of rats with relatively enormous doses of pargyline (100 mg/kg) (Table 2), the suggestion of Weissbach *et al.*,[22] that a drug-insensitive amine oxidase system may be responsible for such phenomena seems equally likely.

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