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ALTERNATIVE PATHWAYS OF 5-HYDROXYINDOLE METABOLISM—II

THE ROLE TRANSAMINATION IN THE METABOLISM OF D-5-HYDROXYTRYPTOPHAN 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—When D-5-hydroxytryptophan was administered to rats primed with ^{15}N-glutamic acid, a significant atom per cent excess of ^{15}N was detected in the urinary 5-hydroxytryptamine arising from its metabolism. This finding suggests that transamination occurs as one stage in the metabolic pathway although there is insufficient evidence to show whether glutamic acid acts directly as the amino group donor or whether the ^{15}N-label is acquired indirectly from the nitrogen pool.

FOLLOWING some observations in man by Oates and Sjoerdsma,[1] Arendt *et al.*[2] have shown that D-5-hydroxytryptophan (D-5HTP), previously thought to be metabolically inert,[3] is vigorously metabolized in the rat, predominantly to 5-hydroxytryptamine (5HT). The pathway is thought to lie via D-amino acid oxidation to the keto-acid, followed by transamination to the L-amino acid and subsequent decarboxylation; so far, there is little direct evidence to confirm the hypothesis.

The present series of experiments was designed to demonstrate the transamination step in this postulated sequence.

EXPERIMENTAL

D-5HTP was prepared from DL-5HTP (Koch-Light Laboratories Ltd.) by the method of Contractor and Wragg.[4]

^{15}N-Glutamic acid, 33 per cent enriched, was obtained from the Commissariat à l'Energie Atomique, Gif-sur-Yvette.

Two groups of black hooded male rats, strain PVG/C (A.R.C. Unit, Compton, Berks.) weighing approx. 150 g, were injected i.p. with varying amounts (Table 1) of ^{15}N-glutamic acid solution. At specified intervals (Table 1), animals in one group (test) were injected i.p. with 2 mg D-5HTP. Separate 24 hr urine specimens were then collected from all animals as described by Arendt *et al.*[2]

5HT (300 μg), corresponding to the expected excretion of 5HT deriving from the D-5HTP injected into test rats, was added to the urine collection vessels of those not treated with D-5HTP (controls). Urine samples were filtered, adjusted to pH 8·5 with

* 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.

2N-NaOH, diluted to 40 ml with water and run through 1 cm dia. columns of Amberlite CG50 resin, 200–400 mesh (12 g suspended in 0·2 M sodium phosphate buffer, pH 8·5). Columns were eluted in 5 ml fractions with sodium phosphate buffer. 5HT, detected by its characteristic fluorescence in 3N-HCl,[5] was eluted between 100–150 ml. 5HT-containing fractions were combined and desalted by passing through 1 cm dia. columns of Amberlite CG50 resin, 200–400 mesh (10 g H^+ form, in water, pH 6). Columns were washed with 30 ml water and then eluted with N-formic acid in 5 ml fractions. 5HT emerged after the passage of 20–25 ml effluent and its elution was complete after a further 25 ml had run through. Combined 5HT-containing fractions were freeze-dried, transferred in a minimal volume of water to Whatman CC 41 cellulose thin-layer plates (20 × 20 cm) as streaks and developed in butanol:acetic acid:water (12:3:5).[4] 5HT was located by its pink fluorescence in methanol–0·1N-HCl under ultraviolet light.[2] The cellulose layer in this area was removed and extracted with 4 × 1 ml methanol which were pooled, taken to dryness and run once more by the same thin-layer chromatographic procedure. In addition to 5HT, two faint ninhydrin-reacting, Ehrlich-positive spots were persistently present, however many chromatographic purifications were undertaken. As these were also present on chromatograms of pure 5HT standards, they were presumed to be degradation products of 5HT and not contaminants from other potentially nitrogen-containing compounds.

The combined methanol extracts were made up to 5 ml with methanol and 2 × 2 ml portions were separately taken to dryness. The residues were incinerated with 2 ml 50% H_2SO_4 containing 1% (v/v) selenium dioxide and 100 μg nitrogen as ammonium sulphate was added to each. Total nitrogen was estimated on one by a conventional micro-Kjeldahl technique.[6] Using the Markham apparatus, ammonia was distilled over from the other into 2 ml 0·1N-H_2SO_4, which was taken down to a small volume and degassed in a Rittenberg tube with a standard vacuum system. Ammonia was oxidized to nitrogen with excess sodium hypobromite solution.[7]

Nitrogen samples were analysed by mass spectrometer. The ratio of molecules of mass 29 (^{15}N-^{14}N) to those of mass 28 (^{14}N-^{14}N) was determined using the double collector principle.[8] Readings were corrected for "residual mass 28", "residual mass 29" and traces of air. The air correction was calculated from the magnitude of the mass 32 (oxygen) peak. The error in per cent label, calculated from the known error ($\pm$1·13 μg) in the determination of total nitrogen by the micro-Kjeldahl procedure, was not more than $\pm$1·1 per cent in any sample. The limiting value of atom per cent excess which can reliably be detected is considered to be 0·0019 (Francis *et al.*, 1954).[8]

No corrections were made for endogenous 5HT excretion which did not appear to exceed 3 μg/24 hr (2 determinations).

RESULTS

Seven pairs of rats were pretreated with glutamic acid and one animal in each pair (test) was given 2 mg D-5HTP. In every test animal, there was a significant atom per cent excess of ^{15}N in isolated urinary 5HT. Small amounts of ^{15}N were found in isolated material from one control only, indicating that other nitrogen-containing urinary constituents had been excluded by the 5HT purification procedure. Increasing the amount of administered glutamic acid from 20 to 100 mg in the test

animal appeared to increase the label, but further dosage rise had little effect. Injecting D-5HTP simultaneously with glutamic acid or after 30 min made no appreciable difference to the findings.

TABLE 1. RELATIONSHIP BETWEEN DOSE OF ^{15}N-GLUTAMIC ACID, AND OF THE INTERVAL BETWEEN ITS ADMINISTRATION AND D-5-HYDROXYTRYPTOPHAN (D-5HTP) INJECTION, ON THE DEGREE OF ^{15}N-LABELLING OF URINARY 5-HYDROXYTRYPTAMINE (5HT) IN TEST RATS COMPARED WITH CONTROLS NOT GIVEN 5-HYDROXYTRYPTOPHAN

Experiment	^{15}N-glutamic acid dosage H-test and control (mg)	Time between glutamic acid and D-5HTP (2 mg) injection-test (min)	Per cent label of 5HT amine nitrogen-test	Per cent label of 5HT amine nitrogen-control
1	20	5	0·73	0
2	100	0	2·43	0·36
3	100	0	1·92	0
4	100	30	3·09	0
5	100	30	1·12	0
6	140	30	2·07	0
7	168	30	1·87	0

DISCUSSION

The degradation pathway proposed by Oates and Sjoerdsma[1] for D-5HTP, involving initial deamination by D-amino-acid oxidase followed by transamination of the keto-acid thus formed to the L-isomer, is generally accepted as a metabolic route for many D-amino acids in higher animals.[9] Whilst D-amino acid oxidase has been widely studied, little is known about L-5HTP transaminase. It was first detected by Sandler *et al.*[10] in a rat liver fraction and soon confirmed by Spencer and Zamcheck;[11] both groups used α-oxoglutaric acid as an amino group acceptor, so that it seemed reasonable during the present work to use glutamic acid as amino donor to take part in the further metabolism of 5-hydroxyindolepyruvic acid presumably formed from D-5HTP breakdown. Of three brain transaminases studied by Fonnum *et al.*[12] and Tangen *et al.*[13] however, the one with the greatest affinity for L-5HTP demonstrated some preference for oxaloacetic acid as amino acceptor.

Glutamic acid takes part in a large number of transamination reactions.[14] After administration, the ^{15}N-labelled compound rapidly enters the nitrogen pool to label a wide variety of nitrogenous compounds.[15, 16] From the viewpoint of the present experiment, this fact has two main consequences. Firstly, a vigorous purification procedure was necessary for isolated urinary 5HT; that traces of ^{15}N were only present in 5HT from one control rat suggests that the methods employed were adequate. Secondly, the mere presence of ^{15}N in isolated 5HT does not necessarily imply that glutamic acid was the amino donor. Whilst it does suggest that transamination has occurred, the label is just as likely to have been obtained from the nitrogen pool; the present experiments do not give any indication of its direct origin.

These experiments were qualitative rather than quantitative and the amount of ^{15}N-glutamic acid injected was governed to some extent by availability and cost. The different time intervals between ^{15}N-glutamic acid and D-5HTP administration were an attempt to obtain optimum conditions for 5HT labelling but in the event,

did not add appreciably to the information obtained. There is some indication that elevating the amount of glutamic acid injected from 20 to 100 mg increased the per cent label but a further rise in dosage had no further obvious effect. Whilst a significant degree of ^{15}N-labelling of urinary 5HT was obtained in test animals, with glutamic acid acting directly or indirectly as amino donor, it is likely that a greater degree of ^{15}N enrichment would have been obtained if the animals had been primed with donor for a period of days, as in the classical experiments of Schoenheimer *et al.*,[17] prior to D-5HTP injection.

There remains one other possible interpretation of the present findings, that D-5HTP is converted to the L-form by an unknown mechanism, whilst L-5HTP undergoes transamination to its analogous keto-acid, picking up the ^{15}N-label on its reamination to L-5HTP. Whilst this series of reactions is considered unlikely, we hope to test the second stage by substituting L- for D-5HTP in a series of experiments similar to the ones described.

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