5-Hydroxytryptamine Metabolism by the Nuclear Fraction of Rat-Liver Homogenate

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1. The metabolism of 5-hydroxy[1'-¹⁴C]tryptamine creatinine sulphate in the nuclear fraction of rat-liver homogenate was studied. In the incubation mixture five metabolites were found. 2. Two metabolites were not radioactive; one of them was identified as 5-hydroxyindole-3-carboxylic acid and the second tentatively as 5-hydroxyindole-3-aldehyde. 3. 5-Hydroxyindol-3-ylacetic acid, 1'-*N*-acetyl-5-hydroxytryptamine and 5-hydroxytryptophol were not precursors of 5-hydroxyindolealdehyde and 5-hydroxyindolecarboxylic acid. 4. It was shown that the metabolism of 5-hydroxytryptamine in the nuclear fraction involves monoamine oxidase, the precursor of 5-hydroxyindolealdehyde and 5-hydroxyindolecarboxylic acid being most probably 5-hydroxyindol-3-ylacetaldehyde.

5-Hydroxytryptamine metabolism has been investigated at all levels, from the whole animal to the preparation of particular enzymes. In this Laboratory some studies have been done with ¹⁴C-labelled 5-hydroxytryptamine, both on whole rats and liver slices (Keglević *et al.* 1959; Kveder, Iskrić & Keglević, 1962; Kveder, 1963). In an attempt to prepare a suspension of rat-liver cells, a tissue preparation was obtained that metabolized 5-hydroxytryptamine in an unusual way, and some hitherto unknown metabolites were detected. When this preparation was more closely examined it was shown to represent the nuclear fraction of liver homogenate containing only few unbroken cells. Since the results were reproducible and the preparation was easily obtained, it seemed to us worthwhile to direct our efforts towards the identification of the unknown metabolites.

It was shown that the nuclear fraction of rat-liver homogenate is able to metabolize 5-hydroxytryptamine by means of monoamine oxidase, but, unlike the whole body or the liver slices, 5-hydroxyindol-3-ylacetic acid is not formed and the end product is 5-hydroxyindole-3-carboxylic acid.

EXPERIMENTAL

Materials. 5-Hydroxy[1'-¹⁴C]tryptamine creatinine sulphate (Keglević-Brovat, Kveder & Iskrić, 1957), 1'-*N*-acetyl-5-hydroxytryptamine (Desaty, Hadžija, Iskrić, Keglević & Kveder, 1962), 5-hydroxytryptophol (Kveder *et al.* 1962) and 5-hydroxyindole-3-carboxylic acid, m.p. 173–176° (S. Kveder & S. Iskrić, unpublished work), were used throughout. All other compounds were from commercial sources.

Preparation of the nuclear fraction. Adult (aged 3–4 months) albino rats were killed by decapitation. A weighed amount of liver was minced with scissors in cold Krebs–Ringer phosphate, pH 7.4 (Umbreit, Burris & Stauffer, 1949), forced through a fine metal screen (15 meshes/cm.) with a glass pestle and finally filtered through a thick nylon cloth. A 10% suspension was repeatedly centrifuged at 700 *g* for 5 min. and resuspended in Krebs–Ringer phosphate until a clear supernatant was obtained. All the above steps were carried out at 0–4°.

Microscopic examination showed that, although a good recovery of cell nuclei (80 × 10⁶ to 100 × 10⁶/g. of tissue) was achieved, the preparation also contained erythrocytes, some protoplasmic fragments and few residual unbroken cells.

Incubation. An amount of nuclear sediment corresponding to 2 g. of original tissue was incubated with substrate in 10 ml. of Krebs–Ringer phosphate for 2 hr. at 37–38° in an atmosphere of oxygen, with shaking. At the end of the incubation period, the reaction mixture was diluted with 10 ml. of 0.9% NaCl and centrifuged at 3500 *g* for 5 min. The clear supernatant was treated with deactivated charcoal (0.4 g.) by the procedure of Dalglish (1955). The phenolic eluate (80 ml.) was evaporated *in vacuo* and the residue subjected to paper chromatography.

Paper chromatography. Ascending two-dimensional chromatography (frame technique; Smith, 1962) on Whatman no. 1 paper was used with propan-2-ol–aq. ammonia (sp.gr. 0.88)–water (10:1:1, by vol.) (solvent *A*) as the first and butan-1-ol–acetic acid–water (4:1:5, by vol.) (solvent *B*) as the second solvent. Dry chromatograms were examined under ultraviolet light (2537 Å). They were sprayed for indoles with Ehrlich's reagent [2% (w/v) *p*-dimethylaminobenzaldehyde in 2 *N*-HCl] and xanthidrol [0.2% (w/v) in 95% (v/v) ethanol; before spraying 10 ml. of 12 *N*-HCl was added to 90 ml. of this solution], for phenols with Brentamine reagent [0.1% (w/v) tetrazotized *o*-dianisidine in 50% (v/v) methanol, followed by exposure to

ammonia vapour] and α -nitroso- β -naphthol [0.1% (w/v) in 95% (v/v) ethanol; after drying sprayed with 0.1% (w/v) NaNO_2 in 2 N-HCl], and for aldehydes with 2,4-dinitrophenylhydrazine (saturated solution in 2 N-HCl). The radioactivity was detected by radioautography with X-ray films (Ferrania).

RESULTS AND DISCUSSION

A typical chromatogram prepared from an incubation mixture with 5 mg. of 5-hydroxy[1'- ^{14}C]tryptamine creatinine sulphate as substrate is

Fig. 1. Two-dimensional chromatogram of the incubation mixture of 5-hydroxy[1'- ^{14}C]tryptamine creatinine sulphate with the nuclear fraction of rat-liver homogenate. Experimental details are given in the text, and details for the detection of metabolites are given in Table 1. Solvent A: propan-2-ol-aq. ammonia (sp.gr. 0.88)-water (10:1:1, by vol.); solvent B: butan-1-ol-acetic acid-water (4:1:5, by vol.). 5-HICA, 5-hydroxyindole-3-carboxylic acid; 5-HIAld, 5-hydroxyindole-3-aldehyde. +, Positions of markers: 5-hydroxytryptamine (5-HT); 5-hydroxyindol-3-ylacetic acid (5-HIAA); 5-hydroxytryptophol (5-HTOL). X_1 , X_2 and X_3 , Unidentified compounds. Radioactive spots are hatched.

presented in Fig. 1. Despite the fact that 5-hydroxytryptamine was completely metabolized only traces of 5-hydroxyindolylacetic acid were detected. At least five new spots appeared; their chromatographic characteristics are given in Table 1. None of these metabolites was found either in the urine of rats given 5-hydroxy[1'- ^{14}C]tryptamine creatinine sulphate (Keglević *et al.* 1959) or in the incubation mixture of the same compound with rat-liver slices (Kveder, 1963).

Three of these metabolites were radioactive, but they did not give distinct colour reactions for indoles. Though two of them, X_1 and X_2 , gave positive reactions for phenols, X_3 gave no colour with any of the reagents used. These compounds have not yet been identified.

The two remaining metabolites were not radioactive. Colour reactions indicated that the indole ring bearing the hydroxyl group in the 5-position was still preserved. Because of the positive reaction with 2,4-dinitrophenylhydrazine and the R_F values, one of them was tentatively identified as 5-hydroxyindole-3-aldehyde. The other metabolite was identified as 5-hydroxyindole-3-carboxylic acid, this being confirmed by co-chromatography with an authentic sample.

It seemed to us that α -oxidation and subsequent decarboxylation of 5-hydroxyindolylacetic acid would be the most plausible explanation of the origin of these compounds. However, this was proved not to happen as no traces of 5-hydroxyindolealdehyde or 5-hydroxyindolecarboxylic acid were found after incubation of 3 mg. of 5-hydroxyindolylacetic acid with the nuclear preparation. Therefore further investigations were undertaken to find out the precursor of 5-hydroxyindolealdehyde and 5-hydroxyindolecarboxylic acid in the metabolic pathway of 5-hydroxytryptamine.

So far, the only claimed metabolic pathway of 5-hydroxytryptamine not involving amino oxidation has been the acetylation of the amino group (McIsaac & Page, 1959) on the way to melatonin (Axelrod & Weissbach, 1961; Lerner, Case &

Table 1. Chromatographic characteristics of metabolites from the incubation mixture of 5-hydroxy[1'- ^{14}C]tryptamine creatinine sulphate with the nuclear fraction of rat-liver homogenate

The compositions of solvents and reagents are given in the text. Colours of spots: B, blue; R-V, red-violet; P, pink; Y, yellow. Intensities: ++, very strong; +, strong; +, weak; (+), very weak; -, negative.

Metabolite	R_F values		Radioactivity	Ultra-violet light	Colour reagents				
	Solvent A	Solvent B			Ehrlich	Xanthidrol	Brentamine	α -Nitroso- β -naphthol	Dinitrophenylhydrazine
Compound X_1	0.22	0.38	++	(+) Y	-	+ P	++	+	-
Compound X_2	0.52	0.56	+++	(+) Y	-	+ P	++	+	-
Compound X_3	0.80	0.38	+	-	-	-	-	-	-
5-Hydroxyindole-3-carboxylic acid	0.10	0.72	-	++ B	++ R-V	++ B	++	++	-
5-Hydroxyindole-3-aldehyde	0.85	0.78	-	(+) Y	(+)	++ B	++	++	+++
5-Hydroxyindolyl-3-acetaldehyde semicarbazone	0.55	0.69	+++	(+) Y	+++	++ B	+++	++	-

Takahashi, 1960). *N*-Acetyl-5-hydroxytryptamine (3 mg.) itself when incubated proved to be a poor substrate for the nuclear fraction and remained unchanged.

When the nuclear fraction was preincubated for 10 min. with 1.7 mM-iproniazid, the metabolism of 5-hydroxytryptamine was almost completely suppressed; there appeared only one minor metabolite that was radioactive. The chromatographic properties of this metabolite corresponded to those of 5-hydroxytryptophyl and *N*-acetyl-5-hydroxytryptamine respectively (Kveder *et al.* 1962), but its complete identification has not yet been attempted.

The experiment with iproniazid showed that the formation of 5-hydroxyindolealdehyde and 5-hydroxyindolecarboxylic acid is an enzymic monoamine-oxidase-dependent reaction. The most probable precursor of these compounds seems to be 5-hydroxyindolyl-3-acetaldehyde. In favour of this presumption were also the results of the next experiment in which 5 mg. of 5-hydroxy[1'-¹⁴C]-tryptamine creatinine sulphate was incubated in the presence of 3 mg. of semicarbazide. In this case 5-hydroxytryptamine was almost completely metabolized, but no traces of 5-hydroxyindolealdehyde or 5-hydroxyindolecarboxylic acid were found; a big radioactive spot, presumably the semicarbazone of 5-hydroxyindolylacetaldehyde (Udenfriend, Titus, Weissbach & Peterson, 1956), appeared. Its chromatographic characteristics are given in Table 1.

Because 5-hydroxyindolylacetaldehyde was not available, 5-hydroxytryptophol was used as its possible precursor (Kveder *et al.* 1962). However, when incubated with the nuclear preparation, 5-hydroxytryptophol gave rise neither to 5-hydroxyindolealdehyde nor to 5-hydroxyindolecarboxylic acid.

Indole-3-aldehyde and indole-3-carboxylic acid have been found both in plants (Fawcett, Seeley, Taylor, Wain & Wightman, 1955; Jones & Taylor, 1957) and animals (Morton & Fahmy, 1958; Balakrishnan & Rodnight, 1960), but opinions about their biosynthesis vary. Among others, α -oxidation followed by subsequent decarboxylation of indolylacetic acid (Meyer, 1958; Stutz, 1958; Pilet, 1961) and a degradation of indolylacetaldehyde (Libbert, 1961) have been proposed. Of 5-hydroxyindolealdehyde and 5-hydroxyindolecarboxylic acid, only the latter was hinted to be present in some rat urines (Acheson & King, 1963). Our experiments showed that 5-hydroxyindolealdehyde and 5-hydroxyindolecarboxylic acid, at least under certain conditions, could be formed in the metabolic pathway of 5-hydroxytryptamine in rats. With regard to their biogenesis in the nuclear preparation all our findings support the presumption that 5-hydroxyindolealdehyde is formed by a degradation

of 5-hydroxyindolylacetaldehyde. The inability of 5-hydroxyindolylacetaldehyde to be oxidized to 5-hydroxyindolylacetic acid indicated that the nuclear preparation, though still containing monoamine oxidase, is lacking aldehyde (or alcohol)-dehydrogenase activity. The same may explain the failure of 5-hydroxytryptophol to serve as the precursor of 5-hydroxyindolylacetaldehyde. Whether 5-hydroxyindolecarboxylic acid is derived from 5-hydroxyindolealdehyde enzymically or is an artifact cannot be decided at present.

The nuclear fraction used was not homogeneous, erythrocytes being the most abundant contaminant. To check their metabolic activity labelled 5-hydroxytryptamine was incubated with a blood sample centrifuged from cold Krebs-Ringer phosphate in the same way as the rat-liver homogenate; however, 5-hydroxytryptamine remained completely unchanged. The inactivity of erythrocytes as well as the negligible quantity of other contaminants (protoplasmic fragments and unbroken cells) in our tissue preparation indicates that the nuclei are the factor responsible for the metabolic transformation of 5-hydroxytryptamine.

There is as yet no evidence that 5-hydroxyindolealdehyde or 5-hydroxyindolecarboxylic acid is formed in the metabolic pathway of either endogenous or exogenous 5-hydroxytryptamine *in vivo*. However, rats given 5-hydroxytryptamine labelled in C- ω of the side chain eliminated a small fraction of the radioactivity in expired carbon dioxide (Keglević-Brovat, Supek, Kveder, Iskrić & Kečkeš, 1958); hence one can expect that from the rest of the 5-hydroxytryptamine molecule 5-hydroxyindolealdehyde or 5-hydroxyindolecarboxylic acid were formed.

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