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THE FORMATION OF 5-HYDROXYTRYPTOPHOL IN BRAIN IN VITRO

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Kveder, Iskric & Keglevic (1962) described the formation of 5-hydroxytryptophol from 5-hydroxytryptamine (5HT) *in vivo* in rats. The alcohol was isolated as conjugates from urine, and distinguished from 1'-N-acetyl 5-hydroxytryptamine by paper chromatography. Feldstein & Wong (1965) demonstrated the enzymatic conversion of 5HT to 5-hydroxytryptophol in rat liver. The alcohol appears also to be a major metabolite of the amine in platelets (Bartholini, Pletscher & Bruderer, 1964).

The isolation of the alcohol from bovine pineal gland by McIsaac, Farrell, Taborsky & Taylor (1965) led us to attempt the synthesis of 5-hydroxytryptophol from 5HT in rat and subsequently human brain homogenates.

METHODS

Reagents

5-hydroxytryptamine creatinine sulphate was obtained from Koch-Light Ltd., Colnbrook, and 5-hydroxyindolacetic acid from Roche Products, Hertfordshire. The authenticated sample of 5-hydroxytryptophol was a gift from the National Institutes of Health, Bethesda.

Preparation of brain homogenates

Wistar strain rats of either sex weighing 180–220 g were used. The animals were killed by decapitation and the brain was quickly taken out. After removal of the cerebral hemispheres and cerebellum the remainder of the brain was homogenized in 25 vol. 0.25M sucrose at 4° ; 1 ml. of the homogenate was preincubated in a metabolic shaking incubator for 15 min at 37°. The reaction was started by the addition of 80 μg of 5HT in 1 ml. of 0.5M phosphate buffer, pH 7.4 (Feldstein & Wong, 1965), containing 500 μg of one of the pyridine nucleotide co-enzymes (nicotinamide adenine dinucleotide (NAD), nicotinamide adenine dinucleotide reduced ($NADH_2$), nicotinamide adenine dinucleotide phosphate (NADP) and nicotinamide adenine dinucleotide phosphate reduced ($NADPH_2$)). The reaction was stopped at varying intervals by the addition of 0.1 ml. conc. HCl. Human brain material obtained at necropsy from an adult female was examined in a manner similar to that described above—that is, samples of midbrain and cerebral cortex were dissected, homogenized in 25 vol. 0.25M sucrose at 4° and incubated with 5HT, etc.

Estimation of 5-hydroxytryptophol, 5-hydroxyindol-3-yl acetaldehyde and 5-hydroxyindol-3-yl acetic acid (5HIAA)

The analyses were a modification of the method reported by Feldstein & Wong (1965). Duplicate incubation mixtures were pooled and centrifuged at 3,000 rev/min for 7 min. After decantation the pH of the supernatant was adjusted to 7.4 (glass electrode) using 5N NaOH, and passed through

a 10 × 0.7 cm column of CG−50 ion-exchange resin (B.D.H. Ltd.) in the ammonium form at pH 7.4 prepared as described by Eccleston, Ashcroft, Crawford & Loose (1966). This took up the majority of the residual 5HT. The acid and neutral metabolites were washed through the column with 1 ml. 0.02M pH 7.4 acetate buffer. The effluent was collected, salt saturated and acidified by the addition of 0.1 ml. conc. HCl. The neutral and acidic components were then extracted into 20 ml. freshly distilled peroxide-free diethyl ether. The acid component was back extracted into 5 ml. salt-saturated 0.2M borate buffer, pH 10, and 1 ml. of the extract was used for determination of the acid. To duplicate 1 ml. aliquots of the buffer extract was added 3 ml. N/10 H_2SO_4 and 2 ml. conc. HCl containing ascorbic acid 100 mg/100 ml., for control estimations. The hydroxy-acid was estimated in a Farrand spectrophotofluorimeter with the activation spectrum emission set at 550 mμ. An activation maximum is obtained at 295 mμ. After addition to the ether phase of 0.1 ml. methanol containing 10 mg/ml. ascorbic acid and 0.1 ml. 20% (v/v) acetic acid, the ether was evaporated under a stream of nitrogen and the residue (about 0.1 ml.) applied under nitrogen to Whatman No. 1 paper strips as described by Ashcroft, Eccleston & Crawford (1965). The chromatogram was developed in 8% (w/v) aqueous NaCl: glacial acetic acid, 100:1 by volume in an atmosphere of nitrogen. Initially, excess authentic 5-hydroxytryptophol was added to a sample to act as marker and the spot was developed with cinnamaldehyde reagent. Subsequently, the 5-hydroxyindoles were localized by cutting a 0.5 cm longitudinal strip from one of the paper chromatograms, spraying with acid acetone (conc. HCl:acetone 5% v/v), and viewing under ultraviolet light. 5-hydroxytryptophol separates from 5-hydroxyindolacetaldehyde under these conditions. The 5-OH indoles were assayed by cutting paper strips containing 5-hydroxytryptophol and 5-hydroxyindolacetaldehyde and eluting into 4 ml. 0.1N sulphuric acid. The amount of

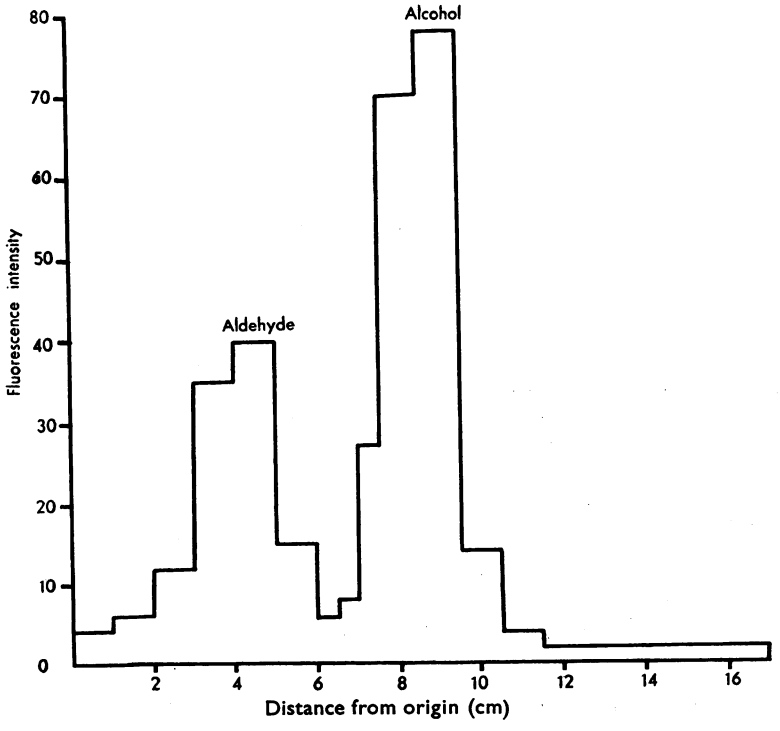

Fig. 1. Separation of 5-hydroxytryptophol and 5-hydroxyindolacetaldehyde by paper chromatography. A sample of the extract containing the neutral indole components was run on Whatman No. 1 paper in the solvent system 8% w/v aqueous NaCl: glacial acetic acid. 100:1. The indoles were assayed after elution from strips cut from the paper, as described in the text.

5-hydroxyindole present in each eluate was determined by spectrophotofluorimetry as described above.

The separation of the compounds was confirmed by taking 4×0.5 cm strips from the paper in the region between the alcohol and the aldehyde (determined under ultraviolet light), eluting into 4 ml. of 0.1N H_2SO_4 and estimating the 5-hydroxyindoles by spectrophotofluorimetry.

Identification of 5-hydroxyindoles

(a) Thin layer chromatography

Extracts of rat brain homogenates after incubation with 5HT and $NADPH_2$ and with the acidic 5-hydroxyindoles removed (as described), were developed in three solvent systems on thin layer plates of silica gel H (Merck) (300μ). The 5-hydroxyindoles were visualized by spraying first with acid acetone and observing under ultraviolet light, followed by spraying with cinnamaldehyde reagent. Comparisons of R_F were made against extracts containing additional authentic 5-hydroxytryptophol. Two major 5-hydroxyindole components were found among the neutral 5-hydroxyindole fractions on thin layer chromatograms. The first behaved like authentic 5-hydroxytryptophol. The second gave a brown colour reaction with the aldehyde spray reagent 2:4 dinitrophenylhydrazine (0.5 g in 100 ml. 2N HCl) and was presumed to be 5-hydroxyindolacetaldehyde. Chromatographic properties of the components are shown in Table 1. The fraction containing acidic 5-OH indoles (pH 10 buffer) was acidified to pH 1, salt-saturated and the acids were extracted into ether. The ethereal extract was taken to dryness after the addition of acetic acid and ascorbic acid, as described above. Chromatography on thin layers of Silica Gel H showed that the single component of the acidic fraction had an R_F value identical with that of authentic 5HIAA.

(b) Spectrophotofluorimetry

The two neutral components were found to have characteristic 5-hydroxyindole fluorescence during an excitation scan showing maximum fluorescence at 295 mμ when emission was set at 550 mμ. Authentic 5-hydroxytryptophol was found to have only 37.3% fluorescence when compared with an equimolar solution of 5HT. (83.3 μg/ml.)

(c) Spectral properties

5-hydroxytryptophol purified and isolated by paper partition chromatography from the neutral fraction of an extract made after incubation was eluted from the paper with spectroscopically pure ethyl alcohol. The ultraviolet spectrum of this solution was examined in a Unicam SP 800 recording spectrophotometer. The E_{max} of this solution at 278 mμ was 5,950 and at 302 mμ, 4,140.

Recoveries (before identification)

Recoveries of 2.5, 5 and 10 μg of authentic 5-hydroxytryptophol taken through the procedure were low (57.2, 42.5 and 44.5% respectively). 5-hydroxytryptophol was not detected in pH 10 buffer used to extract acids. 5HIAA, 10 μg, taken through the procedure gave a recovery of 50.6%. 5HIAA was not detected in the final paper chromatogram. 5HT did not interfere with the estimations at any stage.

pH-Activity Curve

Incubations were continued for 1 hr at 37° in 0.5M phosphate buffer at varying pH values from 5.4 to 9.4 (Fig. 2).

Time course of formation of metabolites

Using optimal pH and coenzyme conditions, incubations were continued over a 4 hr period to determine the curves for the time course of the formation of the 5-OH indole components (Fig. 3).

TABLE 1

THIN-LAYER CHROMATOGRAPHY ON SILICA GEL H OF THE NEUTRAL COMPONENTS IN THE ETHER PHASE

Solvent System: Ethyl Acetate/Chloroform, 3:1 (v/v)

R_F values of components	Acid/acetone reagent + ultraviolet inspection	Cinnamaldehyde reagent	2:4-dinitrophenylhydrazine reagent	Component identified
0·92	—	Deep pink on standing	Yellow on standing	Unknown
0·83	Faint yellow-green fluorescence	Light brown→ blue-grey on standing	Reddish-brown	5-hydroxyindolacetaldehyde
0·5	Yellow-green fluorescence	Green→ blue on standing	Light brown	5-hydroxytryptophol
0·423	—	Pink	Pink	Urea?
0·292	—	Light blue	Faint brown	Unknown
0·15	Yellow-green fluorescence	Very pale purple	Pale yellow	5HIAA as oxidation product? (5HIAA is held at the origin in this solvent)
Solvent System: Ethyl Acetate				
0	—	—	Yellow	Unknown
0·94	—	Deep pink on standing	Yellow on standing	Unknown
0·89	Faint yellow-green fluorescence	Grey-blue	Brown	5-hydroxyindolacetaldehyde
0·7	Yellow-green fluorescence	Grey-blue	Pale orange	5-hydroxytryptophol
0	Faint yellow-green fluorescence	—	Orange	Unknown aldehyde?
Solvent System: iso-Propanol/Methyl Acetate/25% Ammonia, 35:45:20 (v/v)				
1	Faint yellow-green fluorescence	Purple	Yellow	Unknown
0·925	Yellow-green fluorescence	Mauve	Brown	5-hydroxytryptophol
0·85	Yellow-green fluorescence	Blue-grey	Pale brown	5-hydroxyindolacetaldehyde
0·63	Faint yellow-green fluorescence	Pale purple	Yellow	Unknown
0·47	Faint yellow-green fluorescence	Pale green	Yellow	Unknown
0	Faint	—	Yellow	Unknown

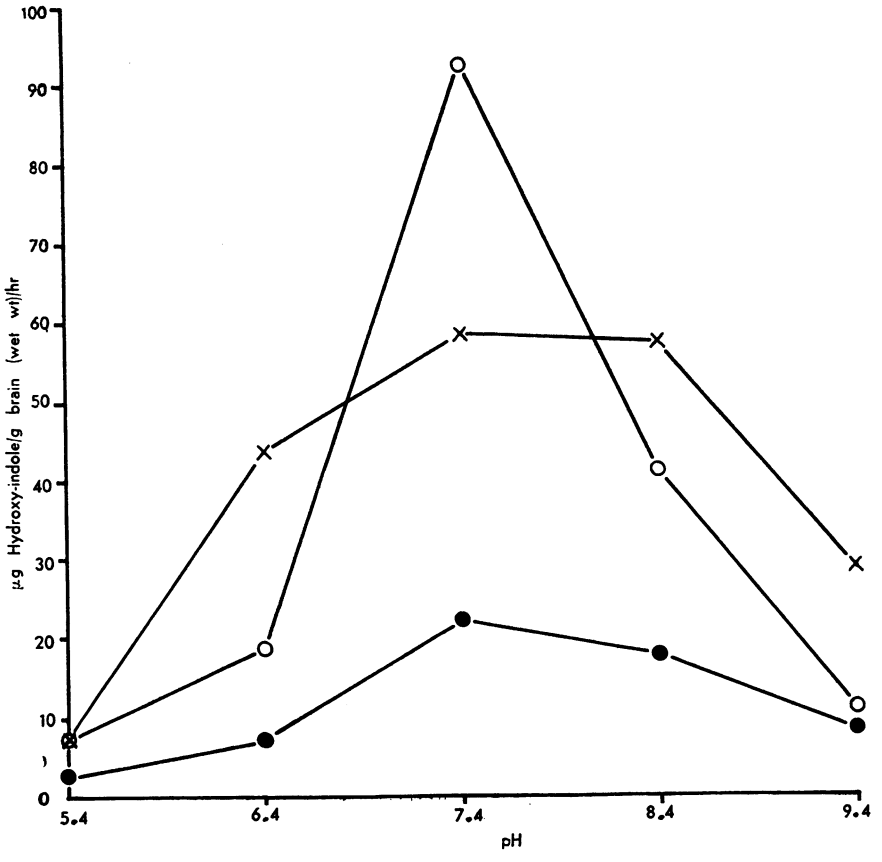

Fig. 2. pH activity curve. 1 ml. samples of rat brain homogenate in 0.25M sucrose were incubated at 37° in the presence of 1 mg $NADPH_2$ and 80 µg 5HT in 1 ml. 0.5M phosphate buffer at the pH values shown. ×——× =5-hydroxyindolacetic acid, ○——○ =5-hydroxytryptophol, ●——● =5-hydroxyindolacetaldehyde.

RESULTS

Enzymatic nature of the formation of 5-hydroxytryptophol

Heating the rat brain homogenate in 0.25M sucrose in a water bath at 100° for 30 min completely abolished its ability to produce 5-hydroxytryptophol from 5HT, even in the presence of excess $NADPH_2$.

Formation of 5-hydroxytryptophol, 5-hydroxyindolacetaldehyde, and 5HIAA in unfortified rat brain homogenates

The amounts of 5-hydroxytryptophol, 5-hydroxyindolacetaldehyde and 5HIAA formed from 5HT, by rat brain homogenates during one hour's incubation are given in Table 2. Without the addition of any exogenous co-factor, the formation of 5HIAA is markedly favoured and the intermediate metabolite, 5-hydroxyindolacetaldehyde, was also produced in considerable amounts.

TABLE 2

CONVERSION OF 5HT TO 5HIAA, 5-HYDROXYTRYPTOPHOL AND 5-HYDROXYINDOLACETALDEHYDE BY RAT BRAIN HOMOGENATES IN THE ABSENCE OF ADDED CO-ENZYME

Results are expressed in μg 5-hydroxyindole formed/g (wet wt.) brain/hr.

Incubation mixture consisted of 1 ml. brain homogenate in 0·25M sucrose + 1 ml. 0·5M phosphate buffer pH 7·4 + 80 μg 5HT. Incubation at 37° C.

Indole	5HIAA	5-hydroxyindolacetaldehyde	5-hydroxytryptophol
Amount	60·32	20·86	1·66

Under such conditions, however, the rate of formation of 5-hydroxytryptophol was very low.

Co-enzyme requirements of rat brain homogenates for the formation of 5-hydroxytryptophol

The pyridine nucleotide requirements for the enzymatic reduction and oxidation of the intermediate 5-hydroxyindolacetaldehyde were investigated by the addition of the oxidized and reduced forms of the pyridine nucleotide co-enzymes to the incubation mixtures of rat brain homogenate and 5HT.

The results are shown in Table 3. The rat brain homogenate can utilize $NADH_2$ slowly for the formation of 5-hydroxytryptophol. Under these conditions, the rate of formation of the alcohol was about twice that in the absence of added co-enzyme. In contrast, 5-hydroxytryptophol formation was markedly stimulated by the addition of $NADPH_2$, indicating that the latter is the preferred co-enzyme.

TABLE 3

FORMATION OF 5HIAA, 5-HYDROXYINDOLACETALDEHYDE, AND 5-HYDROXYTRYPTOPHOL BY RAT BRAIN HOMOGENATES IN THE PRESENCE OF EXCESS PYRIDINE NUCLEOTIDE CO-ENZYME

Results expressed as μg 5-hydroxyindole/g (wet wt.) brain

1 ml. rat brain homogenate incubated at 37° C for times shown in the presence of 80 μg 5HT in 1 ml. 0·5M phosphate buffer pH 7·4 and 1 mg co-enzyme.

		Incubation Time (hr)		
Co-enzyme	5-Hydroxyindole	0	½	1
NADP	5-Hydroxyindolacetic acid	0	61·92	105·24
	5-Hydroxytryptophol	0	3·02	4·43
	5-Hydroxyindolacetaldehyde	0	18·12	17·23
$NADPH_2$	5-Hydroxyindolacetic acid	0	61·59	115·87
	5-Hydroxytryptophol	0	21·30	49·90
	5-Hydroxyindolacetaldehyde	0	12·13	20·65
NAD	5-Hydroxyindolacetic acid	0	50·52	75·94
	5-Hydroxytryptophol	0	1·56	2·09
	5-Hydroxyindolacetaldehyde	0	8·18	13·02
$NADH_2$	5-Hydroxyindolacetic acid	0	45·12	76·50
	5-Hydroxytryptophol	0	2·37	3·35
	5-Hydroxyindolacetaldehyde	0	8·56	12·64

The addition of NADP to the brain homogenates increased the formation of 5HIAA to a greater extent than did the addition of NAD.

Formation of 5-hydroxyindole metabolites by rat brain homogenates with added $NADPH_2$

The formation of the three metabolites produced by rat brain homogenates from 5HT in the presence of excess $NADPH_2$ was followed separately for each component over a prolonged period and the results are given graphically in Fig. 3. These conditions favoured the formation of 5-hydroxytryptophol: the rates of formation of both 5HIAA and 5-hydroxytryptophol were fairly constant up to about 2 hr incubation. It appeared that 5-hydroxyindolacetaldehyde reached a steady state after about 30 min. Incubation for 2–3 hr produced maximum quantities of 5HIAA and 5-hydroxytryptophol, but periods of incubation in excess of 3 hr produced lower yields. This is thought to be due to the decomposition of these unstable compounds.

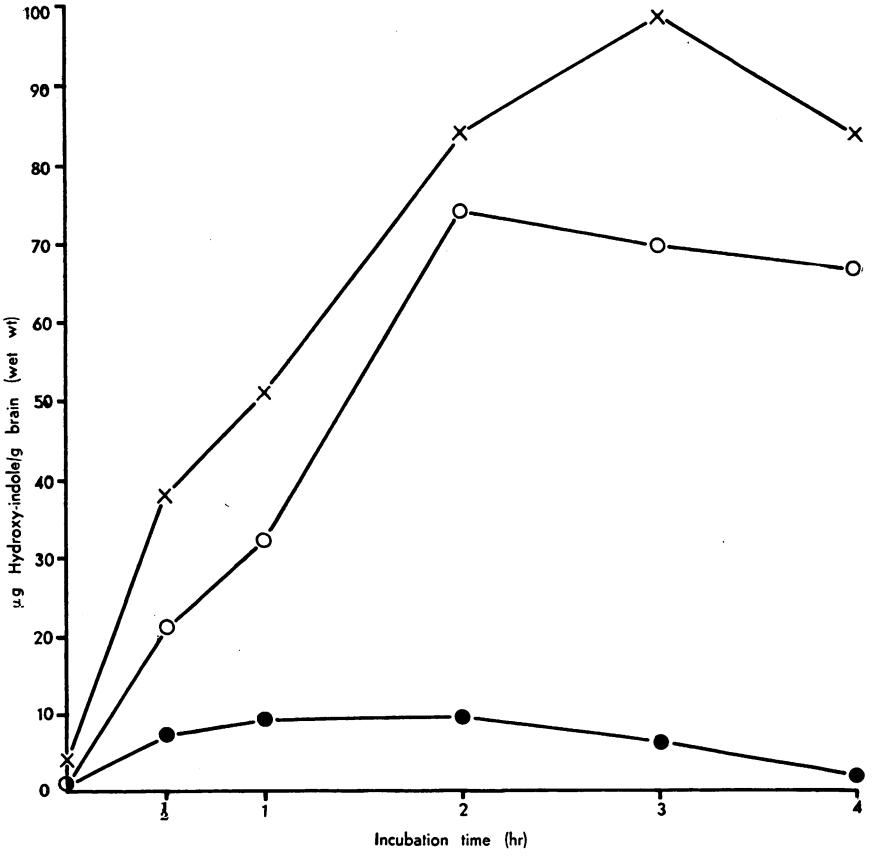

Fig. 3. Time curve for hydroxylating system. 1 ml. samples of rat brain homogenate in 0.25M sucrose were incubated at 37° C in the presence of 1 mg $NADPH_2$ and 80 µg 5HT in 1 ml. 0.5M pH 7.4 phosphate buffer. Samples removed at each time interval and assayed for 5-hydroxyindoles. ×——× = 5-hydroxyindoleacetic acid, ○——○ = 5-hydroxytryptophol, ●——● = 5-hydroxyindolacetaldehyde.

Use of aldehyde " trapping " agents

The addition of the aldehyde " trapping " agents, sodium metabisulphite, and hydroxylamine to the incubation mixtures resulted in an alteration in the relative yields of the 5-hydroxyindole metabolites of 5HT.

The proportion of 5-hydroxyindolacetaldehyde was increased in relation to both 5HIAA and 5-hydroxytryptophol. However, both metabisulphite and hydroxylamine, especially the latter, produced an inhibition of the overall reaction sequence (Table 4).

TABLE 4

ALDEHYDE TRAPPING

Incubation of 1 ml. rat brain homogenate in 0·25M sucrose with 80 μg 5HT in 1 ml. 0·5M phosphate buffer pH 7·4 and 1 mg. $NADPH_2$ with the addition of aldehyde "trapping" agents—that is, sodium metabisulphite (0·12 mg/ml. final concn.) and hydroxylamine hydrochloride (20 μg/ml. final concn).

Results expressed as μg 5-hydroxyindole formed/g (wet wt.) brain/hr.

The aldehyde oxime and sulphite compounds were decomposed by the addition of concn. hydrochloric acid (final HCl concentration, 1*N*) and warmed at 55° C for 10 min before extraction procedure. The 5-hydroxyindole compounds were assayed as in the Methods section

	5-Hydroxy-indolacetic acid	5-Hydroxy-tryptophol	5-Hydroxy-indolacetaldehyde
Control (No "trapping" agent added)	87·18	48·62	12·08
Sodium metabisulphite	69·74	16·68	24·29
Hydroxylamine Hydrochloride	62·86	34·47	13·82

Reversibility of the oxidation-reduction reactions

The use of 5-hydroxytryptophol and 5HIAA as substrates for rat brain homogenates showed that the oxidation of 5-hydroxyindolacetaldehyde to 5HIAA was initially non-reversible, whereas the reduction of 5-hydroxyindolacetaldehyde to 5-hydroxytryptophol was reversible if excess NADP was present.

Formation of 5-hydroxytryptophol, 5-hydroxyindolacetaldehyde and 5HIAA by human brain homogenates

Table 5 gives the results of incubation of sucrose homogenates of human brain samples with 5HT and excess pyridine nucleotide co-enzyme. The samples were taken from midbrain and cortical areas, and although they were not removed from the brain until about 3 hr after death they showed a very high capacity to form 5-hydroxytryptophol. The rate of formation of 5HIAA was lower than in rat brain homogenates under similar conditions. $NADPH_2$ was, like rat brain, the preferred co-enzyme for the reduction of 5-hydroxyindolacetaldehyde. Cortex showed a much higher activity than midbrain for the overall system.

Test for pharmacological activity of 5-hydroxyindolacetaldehyde and 5-hydroxytryptophol

Chromatographically purified 5-hydroxyindolacetaldehyde and 5-hydroxytryptophol were added (final bath concentrations 87 ng/ml. and 180 ng/ml. respectively) to a rat

TABLE 5

FORMATION OF 5HIAA, 5-HYDROXYINDOLACETALDEHYDE, AND 5-HYDROXYTRYPTOPHOL BY HOMOGENATES OF TISSUE FROM HUMAN MIDBRAIN AND CORTEX

1 ml. samples of human brain homogenates were incubated for 1 hr at 37° in the presence of 1 mg $NADH_2$ or $NADPH_2$ and 80 μg 5HT in 1 ml. 0·5M phosphate buffer pH 7·4. Results expressed as μg 5-hydroxyindole formed/g brain (wet wt.)/hr.

Co-enzyme	$NADH_2$			$NADPH_2$		
5-Hydroxyindole Area of brain	5-Hydroxyindolacetic acid	5-Hydroxytryptophol	5-Hydroxyindolacetaldehyde	5-Hydroxyindolacetic acid	5-Hydroxytryptophol	5-Hydroxyindolacetaldehyde
Midbrain	25·75	5·95	10·35	15·63	82·70	5·92
Cortex	65·63	5·51	15·69	37·38	106·64	7·04

uterus preparation (in oestrus) in de Jalon solution. Activity was not observed with either compound. Under the same conditions, 5HT (final bath concentration 2.5 ng/ml.) produced a maximal contraction.

DISCUSSION

The formation of 5HIAA from 5-HT *via* 5-hydroxyindolacetaldehyde in guinea-pig liver preparations was shown by Weissbach, Redfield & Udenfriend (1957) to be effected by a NAD-dependent aldehyde dehydrogenase. McIsaac & Page (1959) found a further metabolite in rat urine following the administration of 5HT, which they postulated to be 1′-N-acetyl-5-hydroxytryptamine. This was confirmed by Weissbach, Lovenberg, Redfield & Udenfriend (1961) who found a lower output of the metabolite in the urine.

Kveder *et al.* (1962) argued that in the metabolism of some amines, intermediate alcohols could be formed: they therefore re-examined the urine of rats injected with radioactively labelled 5HT. They were able to isolate and characterize the glucuronide of 5-hydroxytryptophol as one of the major urinary metabolites of 5HT and suggested that the similar chromatographic behaviour of 1′-N-acetyl-5-hydroxytryptamine and 5-hydroxytyptophol led to confusion of the compounds.

Feldstein & Wong (1965) showed that rat liver homogenates were capable of converting 5HT into 5-hydroxyindolacetaldehyde, 5HIAA and 5-hydroxytryptophol. They suggested liver as the major site for the conversion of 5HT to 5-hydroxytryptophol. However, urinary 5-hydroxytryptophol could also be partly derived from brain and the present experiments have confirmed this possibility and have shown that both rat and human brain homogenates possess the enzymatic mechanism to convert 5HT to 5-hydroxytryptophol, 5HIAA and 5-hydroxyindolacetaldehyde.

The synthesis of 5-hydroxytryptophol in rat and human brain is effected by an alcohol dehydrogenase which has a much higher affinity for NADP/$NADPH_2$ than for NAD/$NADH_2$. Feldstein & Wong (1965) found $NADH_2$ to facilitate the formation of 5-hydroxytryptophol by liver alcohol dehydrogenase, and NAD to facilitate the liver aldehyde dehydrogenase for the formation of 5HIAA. The present experiments show that the aldehyde dehydrogenase of brain has similar co-enzyme requirements for both NAD and NADP, but the specific requirements of brain alcohol dehydrogenase for

$NADP/NADPH_2$ distinguish this enzyme from the corresponding liver enzyme. These differences in co-enzyme specificity have a parallel in liver and retinal alcohol dehydrogenases (Zachman & Olson, 1961; Futterman, 1963).

The pharmacological significance of the complete pathway involving the formation of 5-hydroxyindolacetaldehyde and 5-hydroxytryptophol is not understood. Neither compound exhibits the activity of 5HT on the rat's uterus but, since the equilibrium of the oxidation-reduction phases can be altered by the availability of NADP or $NADPH_2$, situations in which this ratio can be altered by the use of drugs or other agents may be important to brain function. In mammary gland and liver slices, ethanol affects the activity of NADP-dependent enzymes of the pentose phosphate shunt pathway (Beaconsfield & Reading, 1964). A similar situation may occur with the brain 5-hydroxytryptophol-synthesizing system.

The results of adding aldehyde "trapping agents" to the incubation system strongly support the idea that 5-hydroxyindolacetaldehyde, the primary product of monoamine oxidase action on 5HT, is the branching point for the two pathways of reduction and/or oxidation. That such a process takes place in brain is of special importance, since an inhibition of either of the branches might cause a local increase in the concentration of 5HT.

The pH optimum for the complete system appears to be in the region of pH 7 to 8, and the involvement of $NADP/NADPH_2$ in the formation of 5-hydroxytryptophol suggests that the reduction takes place in the soluble fraction of the cytoplasm, whereas the oxidation reaction to 5HIAA, which requires either NAD or NADP, could also take place in the particulate fractions associated with the mitochondria. Since the system has so far only been examined in homogenates, in which the normal intracellular spatial arrangements are destroyed, the extent to which the pathway functions *in vivo* is still an open question. However, the present experiments have demonstrated the existence of the necessary enzymes in brain tissue to produce 5-hydroxytryptophol and 5-hydroxyindolacetaldehyde, and that the activity of these pathways, at least *in vitro*, depends on the $NADP/NADPH_2$ ratio.

SUMMARY

1. Human and rat brain homogenates convert 5-hydroxytryptamine into 5-hydroxytryptophol and 5-hydroxyindolacetic acid via 5-hydroxindolacetaldehyde.

2. The pH optimum for the system lies between pH 7 and pH 8.

3. The formation of 5-hydroxytryptophol is effected by an alcohol dehydrogenase which requires reduced nicotinamide adenine dinucleotide phosphate as coenzyme. Without added coenzyme, the rate of formation of 5-hydroxytryptophol is very low.

4. The coenzyme requirements for the brain alcohol dehydrogenase differ from those of the corresponding liver enzyme.

5. The rate of formation of 5-hydroxyindolacetic acid by brain homogenates is increased by both nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate. However, without the addition of coenzyme the brain homogenate still forms 5-hydroxyindolacetic acid in moderate quantity.

6. Neither 5-hydroxyindolacetaldehyde nor 5-hydroxytryptophol showed pharmacological activity on the rat's uterus when compared with 5-hydroxytryptamine.

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