HYDROXYLATION OF TRYPTOPHAN TO 5-HYDROXYTRYPTOPHAN BY BRAIN TISSUE IN VIVO

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Contrary to an earlier report by Dalgliesh and Dutton (1957), Freedland, Wadzinski and Waisman (1961), using supernatant fraction of rat liver, succeeded in demonstrating in vitro hydroxylation of tryptophan (TP) to 5-Hydroxytryptophan (5-HTP). Recently, Renson, Weissbach and Udenfriend (1962), showed that hydroxylation of TP and phenylalanine by liver extracts was catalyzed by the same enzyme; i.e. phenylalanine hydroxylase. These authors, however, questioned whether this enzyme in the liver was responsible for the in vivo hydroxylation of TP, noting that amethopterin, an inhibitor of phenylalanine hydroxylase, did not affect 5-Hydroxytryptamine (5-HT) level of the brain. In support of a tryptophan hydroxylating enzyme of the intestinal mucosa, described by Cooper and Melcer (1961), Weber and Horita (1963), showed an in situ increase of 5-HT in cannulated rat intestine perfused with L-tryptophan. As a further corollary to an extrahepatic hydroxylation of TP to 5-HTP are the observations of Bertaccini (1960), indicating maintenance of normal cerebral 5-HT content for over three days in total gastro-enterectomized rats. These facts, coupled with the rapid turnover of 5-HT in the brain reported by Udenfriend and Weissbach (1958), were suggestive of direct

hydroxylation of TP to 5-HTP in the CNS. In a recent report by Gal and Marshall (1963), certain aspects of the *in vivo* hydroxylation of TP in pigeon brain were presented. The present communication confirms the presence of direct hydroxylation of TP to 5-HTP in both pigeon and rat brain.

Pigeons of 400 gram and male Sprague-Dawley rats of 300 gram weight kept on standard diet with water *ad libitum* were divided into two groups. All animals received intraperitoneal injection of 3.57×10^{-5} moles/kg β-phenylisopropylhydrazine HCl (PIH) (Lakeside Laboratories) two hours before injection of radioactive tryptophan (Tracerlab, Inc.). Animals in one group were given intraperitoneally 1.5 μmoles of DL-tryptophan-2-C^{14} while those in the other group were injected intracerebrally with 0.085 μmoles of DL-tryptophan-3-C^{14}. The animals were sacrificed at various time intervals. Their brains were quickly removed and homogenized in ice cold 0.01 N HCl. A known volume of the homogenates was taken for determination of total radioactivity, while the remainder was extracted with n-butanol by a modified technique of the method by Bogdanski, Pletscher, Brodie and Udenfriend (1956). Aliquots of the butanol extracts were treated with 3 N HCl from which 5-HT was determined by spectrophotofluorometric assay while from the bulk of the butanol layer 5-HT was removed with 0.01 N HCl. The acid extracts were adjusted to pH 4-5 and lyophylized to dryness. The dry residues were extracted with 80% acetone and the extracts after concentration to 0.2 - 0.5 ml volume were spotted without heat drying on Whatman No. 1 paper, were developed by ascending technique of chromatography in three different solvents, isopropanol:ammonium hydroxide:water (20:1:2), aqueous KCl 20%, or in n-butanol:acetic

acid:water (8:1:1), for 24 hours. After air drying, the chromatograms were exposed to x-ray films (Eastman-Kodak No-Screen) for two weeks. The 5-HT spots were identified by their R_f values compared to a 5-HT-C^{14} (New England Nuclear Corp.) marker. In some experiments, the radioactive spots were cut out and were counted by liquid scintillation technique according to Takahashi, Hattori and Maruo (1961).

In several instances, the spots corresponding to radioactive 5-HT were eluted with 0.01 N HCl. The eluted product showed colorimetric and fluorescent properties similar to authentic 5-HT.

Biological assay of the eluate, using rat stomach muscle strips according to the method of Vane (1957), confirmed the presence of 5-HT. In other experiments, the eluate was neutralized and incubated for an hour in a system containing guinea pig liver mitochondria and kidney aldehyde dehydrogenase to accomplish oxidation of any 5-HT to 5-hydroxyindolacetic acid (5-HIAA). The radioactive product was then extracted according to the method of Udenfriend, Weissbach and Clark (1955) and identified by fluorometric, radioautographic and colorimetric analyses as labeled 5-HIAA. A final identification of the label in 5-HT was proved by recovering the amine after addition of carrier 5-HT as its picrate salt. The picrate salt was recrystalize to constant specific activity. From this picrate salt 5-HT was recovered as its hydrochloride and was counted in the liquid scintillation counter.

Since tryptamine is not hydroxylated to form 5-HT or 5-HIAA, as shown by the experiments of Udenfriend, et. al. (1959), the appearance of label in 5-HT following intracerebral injection of radioact

tryptophan was accepted by us as an evidence of hydroxylation of tryptophan to 5-HTP, the metabolic precursor of 5-HT.

The results in Table I indicate that following I.P. injection o labeled tryptophan, less than 1% of the total radioactivity penetrat into the brain with none of it appearing in the cerebral 5-HT. Ther was, however, a consistent recovery of radioactive 5-HT from the bra within a sixty minute period in the PIH treated animals when labeled tryptophan was intracerebrally administered. Radioautography of the brain extracts unequivocally recorded the presence of labeled 5-HT and tryptamine.

TABLE I

EVIDENCE OF CONVERSION OF TRYPTOPHAN TO 5-HYDROXYTRYPTOPHAN BY BRAIN IN VIVO

GROUP[a]	TIME (min)	TOTAL DPM x10^3	TOTAL DPM 5-HT	RADIOACTIVITY 5-HT % [d]	S.A. 5-HT µc/mmole
Pigeon	60[b]	2.8	none	none	----
	20[c]	645.0	2288	0.50	45.9
Rat	30[b]	3.2	none	none	----
	30[c]	208.0	792	0.18	40.2

[a] All animals received intraperitoneal injection of PIH (3.57×10^{-5} moles/kg) 2 hours prior to injection of tryptophan.
[b] Intraperitoneally administered DL-tryptophan-C^{14} (1.5 µmoles).
[c] Intracerebrally administered DL-tryptophan-C^{14} (0.085 µmoles).
[d] Calculation was based on the assumption that only the L-isomer was hydroxylated.

Without PIH pretreatment, radioactive 5-HT could not be demonstrated in the brain of animals after twenty minutes following intracerebral injection of labeled tryptophan.

In all, these results indicate a direct hydroxylation of TP to 5-HTP by the brain tissue of pigeons and rats.

Details of this work will appear elsewhere.

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