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July 28, 1969

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The formation of 5-hydroxytryptophol from exogenous 5-hydroxytryptamine in cat spinal cord *in vivo*

5-Hydroxytryptophol was identified in 1962 as one of the main metabolites of exogenous 5-hydroxytryptamine (5-HT) in rats (Kveder, Iskrić & Keglević, 1962). Its occurrence in human urine (Davis, Cashaw & others, 1966) shows that it is also a metabolite of endogenous 5-HT in man. However, in the central nervous system—where the metabolism of 5-HT is quite vigorous (cf. Bulat & Supek, 1968)—5-hydroxytryptophol has been found, so far, only in the pineal body (McIsaac, Farrell & others, 1965). Several authors (Feldstein & Wong, 1965; Eccleston, Moir & others, 1966) have shown that the rat brain tissue is able to metabolize 5-HT into 5-hydroxytryptophol *in vitro*. The present communication reports the formation of 5-hydroxytryptophol from 5-HT in the spinal cord *in vivo*.

We have chosen the spinal cord for studying the metabolism of 5-HT because lumbar and sacral cord show the highest density of 5-HT nerve terminals in mammalian central nervous system (Fuxe, Hökfelt & Ungerstedt, 1969). The experiments were made with adult cats lightly anaesthetized with thiopentone sodium. The lumbosacral cord was exposed and two fine polyethylene tubes were inserted subarachnoidally, one at L_1 segment (inflow) and the other at S_4 segment (outflow). A closed subarachnoid space was formed by tying the thread around dura at L_1 and S_4 segment. To remove cerebrospinal fluid the subarachnoid space was first washed with 5-HT creatinine sulphate in Krebs-Ringer buffer (1 mg of free base/ml), and then it was filled with the same solution (*ca* 0·5 ml) which was left in contact with the spinal tissue for 90 min. After, the solution was collected and the subarachnoid space washed with 2 ml of Krebs-Ringer buffer. Then, both solutions ("superfusate") were pooled and deproteinized with perchloric acid. The portion of the spinal cord from L_1 to S_4 segment (about 2·27 g) was dissected, dura and arachnoid stripped off, spinal tissue washed with Krebs-Ringer to remove the adsorbed 5-HT and homogenized with 3 volumes of 0·5M perchloric acid.

Table 1. *5-Hydroxyindoles found in tissue and superfusate after 90 min exposure of the spinal cord segment (approximately 2·27 g) to 5-HT solution (0·5 ml; 1 mg free base/ml Krebs-Ringer buffer) applied subarachnoidally. The amounts are expressed as μg/g of wet tissue*

5-Hydroxyindoles	Spinal tissue		Superfusate	
	Cat I	Cat II	Cat I	Cat II
5-Hydroxytryptamine	5·13	12·92	40·52	53·09
5-Hydroxyindoleacetic acid ..	3·64	4·03	2·53	4·20
5-Hydroxytryptophol	0·48	0·64	0·69	0·84

The excess of perchloric acid from superfusate and homogenate was removed with KOH, both fractions evaporated to a small volume and subjected for qualitative analysis to thin-layer chromatography on silica gel G. The chromatograms were sprayed with Ehrlich reagent for the detection of indolic compounds. Besides unmetabolized 5-HT and 5-hydroxyindoleacetic acid, 5-hydroxytryptophol was detected and its identity confirmed by chromatography with an authentic sample [Rf −0·66; 0·81; 0·46 in solvents: chloroform : acetic acid : methanol (75 : 5 : 20); isopropanol : ammonia : ethyl acetate (35 : 20 : 45); ethyl acetate : chloroform (75 : 25)].

Deproteinized superfusate and homogenate were also subjected to successive column chromatography for the separation of 5-HT and its metabolites as described previously (Iskrić, Stančić & Kveder, 1969) and the indolic compounds were quantitatively determined by spectrofluorometry. Besides 5-HT, its two metabolites—5-hydroxyindoleacetic acid and 5-hydroxytryptophol—were found in measurable quantities. The results of two typical experiments are given in Table 1. To assure that the fluorescence measured is really caused by 5-hydroxytryptophol, the appropriate fractions were pooled and subjected to thin-layer chromatography. The spot assigned to 5-hydroxytryptophol was chromatographically identical to that of the authentic sample.

These preliminary experiments show that the described preparation of the cat spinal cord is a practical and simple experimental model for studying 5-HT metabolism in the central nervous system *in vivo*, since fairly large amounts of the substrate, needed for the detection of some metabolites, can be applied. Such large amounts applied in the brain would cause toxic or lethal effects.

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October 20, 1969

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