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ACTIVATION OF TRYPTOPHAN HYDROXYLASE BY STIMULATION OF CENTRAL SEROTONERGIC NEURONS

Margaret C. Boadle-Biber,* Jan N. Johannessen,* Nedathur Narasimhachari+ and Tam-Hao Phan*

Departments of *Physiology and +Psychiatry, Medical College of Virginia,

Richmond, VA 23298, U.S.A.

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Electrical stimulation of serotonergic neurons in the raphe nuclei of the midbrain or medulla enhances the formation of serotonin *in vivo* in the terminal projections of these neurons [1]. The acceleration of serotonin synthesis results from an increase in the conversion of tryptophan to 5-hydroxytryptophan (5-HTP) by tryptophan hydroxylase [2,3] [EC 1.14.16.4 tryptophan-5-monooxygenase: L-tryptophan, tetrahydropteridine: oxygen oxidoreductase (5-hydroxylating)], the initial and rate-limiting enzyme in the reaction sequence leading to the formation of serotonin. It is likely that this increase in serotonin synthesis is due to an activation of tryptophan hydroxylase itself since this has previously been shown to occur with *in vitro* depolarization of rat brain slices [4-7]. Earlier attempts to demonstrate an increase in tryptophan hydroxylase activity *in vitro* after *in vivo* electrical stimulation of serotonergic nuclei have, however, been unsuccessful [3]. We now present preliminary evidence that *in vivo* electrical stimulation of the serotonergic dorsal raphe nucleus produces an increase in the activity of cortical tryptophan hydroxylase, measured *in vitro*, that parallels increases both in the *in vivo* conversion of tryptophan to 5-HTP (in the presence of an inhibitor of aromatic amino acid decarboxylase) and in the accumulation of 5-hydroxyindoleacetic acid (5-HIAA), the metabolite of serotonin.

In these experiments, male Sprague-Dawley rats (450-550 g) were anesthetized with chloral hydrate (400 mg/kg, i.p.) and placed in a small animal stereotaxic apparatus (David Kopf Instruments). Stimulating electrodes (size 00 insect pins from Carolina Biological Supply), insulated to within 0.5 mm of the tip, were positioned in the dorsal raphe nucleus according to coordinates from the atlas of Pellegrino *et al.* [8] (anterior-posterior, +0.3 mm; lateral, 0 mm; depth, -1.3 mm). The animals were grounded by a probe in the rectum. Stimulation consisted of constant current monopolar square waves (200 uamps, 1.5 msec pulse width, 10 Hz) delivered from a Grass S-48 stimulator coupled to a Grass Constant Current Unit. Experimental animals received 30 min of continuous stimulation while shams were unstimulated. In one group of animals *in vivo* accumulation of 5-HTP was measured in order

to assess the effectiveness of the nerve stimulation (Experiment I, Table 1). These animals were injected with the aromatic amino acid decarboxylase inhibitor, benserazide (seryltrihydroxybenzyl hydrazine, Ro4-4602, Hoffmann-LaRoche, Nutley, NJ) (800 mg/kg, i.p.) 5 min before the start of stimulation (or sham stimulation). In a second group of animals, the effectiveness of nerve stimulation was monitored by the increase in 5-HIAA accumulation in stimulated over sham-stimulated controls. These animals were not treated with benserazide (Experiment II, Table 1). At the end of the stimulation, the animals were rapidly removed from the stereotaxic and decapitated. The right and left cerebral cortices were dissected free, frozen on solid carbon dioxide, and stored at -30°C. The preparation and assay of tryptophan hydroxylase were carried out the next day on the right cerebral cortex according to published procedures [5-7], except that the tissue was homogenized in 2 vol. of 0.05 M Tris acetate buffer (pH 7.4) and the low speed supernatant fraction, obtained by centrifugation of the homogenate at 39,000 g (18,000 rpm) for 30 min, was not passed over Sephadex G-25. Tryptophan hydroxylase activity was determined in the presence of a subsaturating concentration (50 μM) of the artificial reduced pterin cofactor, 6-methyl-5,6,7,8-tetrahydropterin ($6MPH_4$) and a barely saturating concentration (200 μM) of L-tryptophan [5-7]. The 5-HTP formed in the assay was quantitated by high performance liquid chromatography (HPLC) with electrochemical detection on a Bioanalytical Systems Liquid Chromatograph, model 304. The amperometric detector (model LC-4) was set at 0.65 V, and had a glassy carbon electrode. The column was a Waters Radial Compression Module, RCM 100, with a 10 μm, C18 cartridge and the mobile phase, 0.34 M ammonium acetate, pH 5.35, in 5% aqueous methanol. The 5-HTP eluted between two large peaks due to the pterin cofactor (see Fig. 1). The identity of the 5-HTP peak was established by spiking the sample with standard 5-HTP. Enzyme activity is expressed as ng 5-HTP formed per mg protein per min ± S.E.M.

Analysis of 5-HIAA or 5-HTP (in the case of the benserazide-treated animals) was also carried out by HPLC on extracts of left cerebral cortex [9,10]. The tissue was homogenized with an Ultraturrax homogenizer in 2 vol. of chromatographic buffer (0.34 M ammonium acetate, pH 5.35, in 5% aqueous methanol) containing 200 ng/ml of 5-hydroxyindole (5-HI) as internal standard and 1.0 mM tranylcypromine (Parnate, Smith, Kline & French, Philadelphia, PA) to inhibit monoamine oxidase activity. The clear supernatant fraction obtained after centrifugation of the homogenate at 18,000 rpm for 10 min was injected directly onto a μBondapak C18, 10 μm column (0.4 x 30 cm) from Waters Associates. A guard column packed with pellicular (30-38 μm) C18 media (Whatman) was used to protect the μBondapak column (and also the RCM 100 cartridge). The flow rate was 0.8 ml/min and the oxidation potential was 0.65 V. Retention times, in minutes, were: 5-HTP, 8.1; 5-HIAA, 10.8; 5-HT, 12.6; and 5-HI, 15.6. The tissue content of 5-HTP or 5-HIAA was calculated from the ratios of the peak heights of 5-HTP or 5-HIAA to the internal standard in the samples and a standard mixture and is

TABLE 1. Effect of electrical stimulation *in vivo* on *in vitro* and *in vivo* tryptophan hydroxylase activity and levels of 5-HIAA in rat cerebral cortex*

	Tryptophan hydroxylase activity				5-HIAA	
	In vitro		*In vivo*			
	(ng 5-HTP/mg/min ± S.E.M.)	N	(ng 5-HTP/g ± S.E.M.)	N	(μg/g ± S.E.M.)	N
Experiment I						
Sham-stimulated	12.7 ± 0.4	3	146 ± 23	3	---	
Stimulated	22.9 ± 2.5 (+ 80%)	3	367 ± 11 (+ 151%)	3	---	
	P < 0.02		P < 0.001			
Experiment II						
Sham-stimulated	11.9 ± 0.3	4	---		0.59 ± 0.02	4
Stimulated	21.1 ± 1.0 (+ 77%)	4	---		1.17 ± 0.09 (+ 98%)	4
	P < 0.001				P < 0.001	

*N = the number of animals tested. P values indicate the significance of the difference between stimulated and sham-stimulated groups, determined by Student's *t* test.

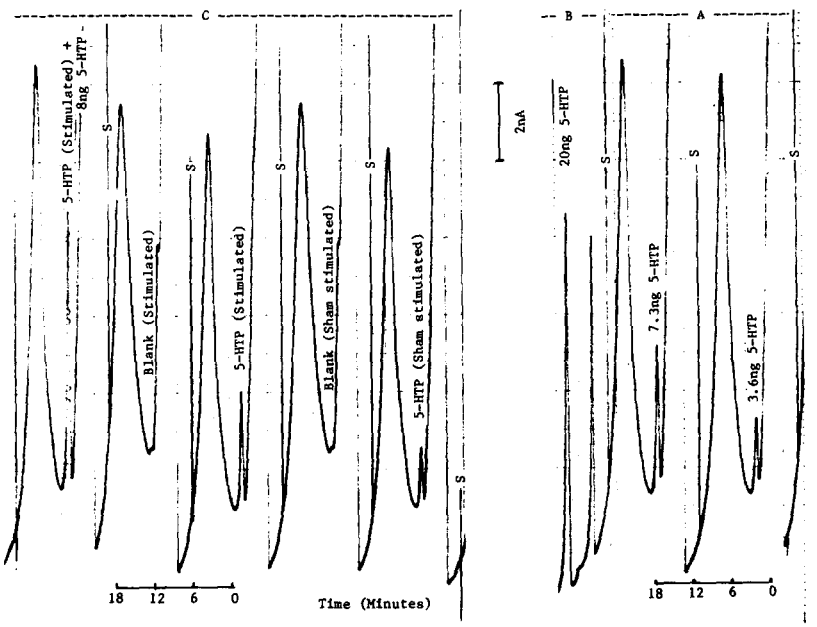

Fig. 1. Chromatogram showing the separation of 5-HTP formed in the *in vitro* assay of tryptophan hydroxylase. A low speed supernatant preparation of tryptophan hydroxylase (100 μl) was incubated for 10 min in the presence of 200 μM L-tryptophan and 50 μM $6MPH_4$ in a total reaction volume of 300 μl. The reaction was terminated with 30 μl of 70% perchloric acid, precipitated protein was removed by centrifugation and 20 μl of the resulting clear supernatant fraction was injected onto the column. Blanks were run with D- instead of L-tryptophan. (A) 5-HTP standards incubated in the presence of assay reagents, but no enzyme. (B) 5-HTP alone. (C) Assay of enzyme from right cerebral cortex of one stimulated and one sham-stimulated animal. Chromatographic conditions: column, Waters RCM 100 10 μm C18 cartridge; mobile phase, 0.34 M ammonium acetate buffer, pH 5.35, in 5% aqueous methanol; flow rate, 0.7 ml/min; and oxidation potential, 0.65 V. S indicates sample injection.

expressed as ng per g fresh tissue ± S.E.M.

The electrical stimulation carried out in the two experiments illustrated in Table 1 was highly effective for, in Experiment I, there was a 150% increase in the in vivo measure of tryptophan hydroxylase activity, and in Experiment II, the levels of the serotonin metabolite, 5-HIAA, doubled. When tryptophan hydroxylase activity was assayed in vitro there was a highly significant increase in enzyme activity in both experiments (Table 1 and Fig. 1). This finding was unexpected in view of the absence of a similar increase in the activity of enzyme from spinal cord under apparently similar conditions of stimulation [3]. Experiments are currently in progress to try to determine whether there are differences in the regulatory properties of the enzyme from these two regions of the central nervous system, namely spinal cord and cerebral cortex. In addition, a detailed study is underway to determine the sensitivity of this increase in cerebral cortical enzyme activity to frequency and duration of stimulation, and to identify the changes in the kinetic properties of the enzyme that occur with stimulation. It will be of interest to determine whether they are similar to the changes reported for enzyme from depolarized slices of brain stem [4-6] or for enzyme activated by incubation in the presence of phosphorylating conditions [11].

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