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Tryptophan Hydroxylase: Activation In Vivo Following Stimulation of Central Serotonergic Neurons

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Previous studies have shown that changes in impulse flow within central serotonergic neurons alter serotonin turnover. In vivo stimulation of the serotonergic perikarya in the midbrain raphe nuclei increases forebrain serotonin synthesis (1) and metabolism (2,3). Conversely, interruption of impulse flow by acute raphe lesion or brain hemisection decreases serotonin synthesis and metabolism in the forebrain (4,5). These changes in serotonin biosynthesis produced by changes in impulse flow may be mediated by an alteration in the activity of tryptophan hydroxylase, the enzyme catalyzing the rate limiting step in the serotonin synthetic pathway.

Two major mechanisms have been implicated in the regulation of tryptophan hydroxylase activity. It has been suggested that tryptophan hydroxylase may be controlled, at least in part, by an end product inhibitory mechanism (6,7). However, this mechanism has not been universally accepted (8). There is also evidence suggesting that the availability of the precursor, tryptophan, may be important in regulating tryptophan hydroxylase activity. Brain tryptophan hydroxylase has a Km for its substrate which is similar to the concentration of endogenous tryptophan found in brain tissue. Thus, under normal conditions the enzyme is not saturated and it has been shown that changes in serotonin synthesis produced by certain drugs in vivo are accompanied by parallel changes in levels of endogenous tryptophan (9). Stimulation of central serotonergic

neurons, however, is not associated with changes in brain tryptophan (1). Thus, neither of these mechanisms can adequately explain the changes in transmitter synthesis which occur in response to alterations in neuronal impulse flow.

Catecholamine biosynthesis in the brain is also influenced by changes in neuronal activity. Recent studies on tyrosine hydroxylase, an enzyme with many properties in common with tryptophan hydroxylase, have shown that this enzyme is activated by periods of increased impulse flow. This activation of tyrosine hydroxylase outlasts the period of neuronal stimulation and appears to occur as a result of kinetic alterations of the enzyme which can be demonstrated *in vitro* following the termination of the stimulation period (10,11). In view of these observations it was of interest to determine if stimulation of central serotonergic neurons results in a similar increase in tryptophan hydroxylase activity which persists beyond the period of neuronal stimulation. If such an activation of tryptophan hydroxylase did occur it would then be possible to investigate the mechanism involved in this stimulus induced activation *in vitro*. The following is a preliminary report of our attempts to demonstrate an *in vivo* activation of tryptophan hydroxylase following electrical stimulation of central serotonergic neurons.

In these experiments serotonergic fiber tracts which project to terminals in the forebrain were electrically stimulated just anterior to their origin in the midbrain raphe. Following the termination of stimulation *in vivo* tryptophan hydroxylase activity was determined by measuring the accumulation of 5-hydroxytryptophan (5HTP) 30 minutes after injection of a central aromatic amino acid decarboxylase inhibitor, Ro4-4602 (seryltrihydroxybenzyl-hydrazine, 800 mg/kg), according to the method of Carlsson and Lindqvist (12).

Male Sprague Dawley rats (250-300 g) obtained from Charles River Breeding Laboratories were anesthetized with chloral hydrate (400 mg/kg, i.p.) and placed into a stereotaxic apparatus. A bipolar coaxial stimulating electrode with a tip separation of 1.5 was lowered 9.0 mm from the surface of the skull, 0.8

mm anterior to lambda (the intersection of the sagittal and lambdoid sutures). The stimulus was provided by a Grass model 5 stimulator with a constant current source of 200 μ amps. Stimulating pulses of alternating polarity and total duration of 3.0 msec were applied at a frequency of 10/second for a total of 30 minutes. In sham operated controls, electrodes were similarly placed but no current was passed. Five minutes after the termination of the stimulation period rats were injected with Ro4-4602 (800 mg/kg, i.p.) and sacrificed 30 minutes later. Forebrain tissue was isolated by a cut from the rostral border of the superior colliculi to the caudal border of the hypothalamus. The tissue was frozen on dry ice, weighed and stored at -70°C until used for assay of tryptophan and 5HTP. The remainder of the brain tissue was fixed in 5% gluteraldehyde-normal saline and 50 u frozen sections were cut and stained with cresyl violet for histological verification of electrode placement. Tryptophan and 5HTP were separated from 5HT and 5HIAA by a modification of the method of Lindqvist (13). Fluorescence of 5HTP was determined after reaction with o-phthalaldehyde by the method of Korf and Valkenburgh-Sikkema (14). Fluorescence of endogenous tryptophan was determined after conversion to norharman by the method of Hess and Udenfriend (15).

Figure 1 shows that the accumulation of 5HTP in the forebrain of unstimulated rats is linear with time for at least the first 45 minutes after injection of Ro4-4602. In the 30 minute period beginning 5 minutes after the end of neuronal stimulation there was an 83% increase in drug induced accumulation of 5HTP in stimulated rats compared to sham operated controls (Table 1). Levels of endogenous tryptophan were unchanged under these conditions.

These results indicate that the increase in forebrain tryptophan hydroxylase activity produced by stimulation of fiber tracts originating in the midbrain raphe and projecting to the forebrain outlasts the period of neuronal stimulation. This increase in <u>in vivo</u> enzyme activity appears to be unrelated to levels of precursor although the occurrence of small localized changes in tryptophan concentrations cannot be entirely excluded by our methods.

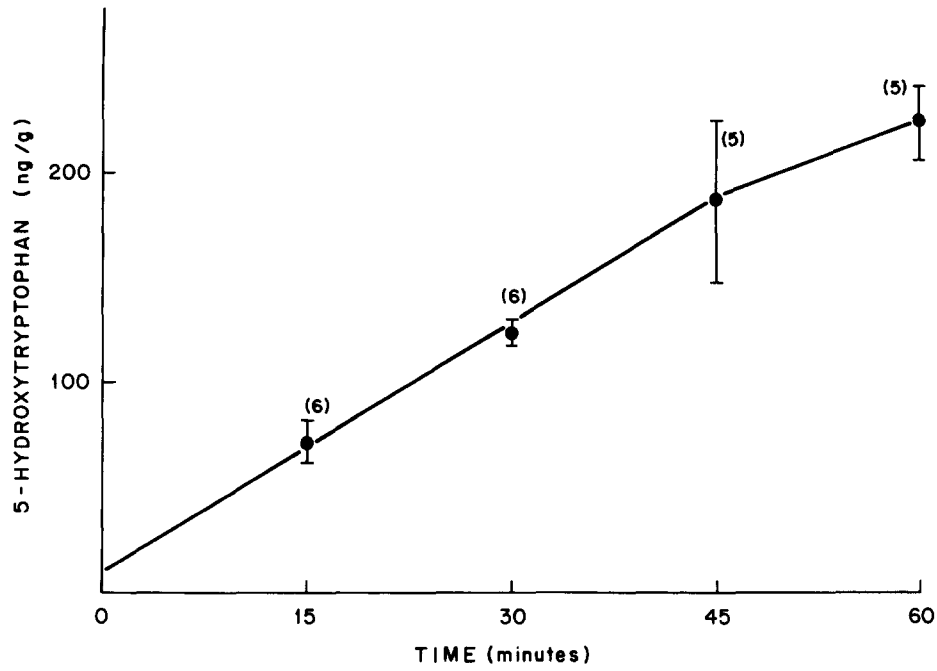

Figure 1. Time course of 5-hydroxytryptophan (5HTP) accumulation in the rat forebrain after Ro4-4602. Rats were sacrificed at various time intervals after injection of Ro4-4602 (800 mg/kg, i.p.). Forebrains were analyzed for 5HTP. The number of rats in each group is indicated in parentheses. The line indicating the accumulation of 5HTP up to 45 minutes was calculated by the method of least squares. Analysis of variance shows that points are significantly different for the first 45 minutes, $p < .01$.

TABLE 1

Ro4-4602 Induced Accumulation of 5HTP in Rat Forebrain After Raphe Stimulation of Central Serotonergic Neurons

Treatment	n	Tryptophan (μg/g)	5HTP (ng/g)	% change 5HTP
Sham Stimulated	6	5.81 ± 0.22	72.5 ± 5.7	
Raphe Stimulated	8	6.33 ± 0.15	132.9 ± 3.6[a]	+83.3

Rats were stimulated for 1/2 hour as described in methods. Five min after termination of the stimulation period all were injected with Ro4-4602 (800 mg/kg, i.p.) and sacrificed 1/2 hour later. Forebrain tissue was analyzed for tryptophan and 5HTP.
[a]Significantly different from sham operated controls, $p < .001$.

In addition, in this experiment tryptophan hydroxylase activity is still activated subsequent to the period of direct neuronal stimulation when transmitter is presumably no longer being released. Since the activation of the enzyme

appears to be independent of serotonin release, this phenomenon cannot be adequately explained by removal of an end product inhibitory mechanism. These data are consistent with the hypothesis that neuronal stimulation results in a direct activation of the enzyme. Experiments are now in progress to determine whether a stimulus-induced kinetic activation of tryptophan hydroxylase is sufficient to explain the increased activity produced by neuronal stimulation.

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