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INCREASED TRYPTOPHAN HYDROXYLASE ACTIVITY IN SEROTONERGIC NERVE TERMINALS SPARED BY 5,7-DIHYDROXYTRYPTAMINE

MICHAL K. STACHOWIAK, EDWARD M. STRICKER, JACOB H. JACOBY and MICHAEL J. ZIGMOND*

Departments of Biological Sciences, Psychology and Psychiatry, Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260, U.S.A.

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Abstract—Adult rats received intraventricular injections of 5,7-dihydroxytryptamine (5,7-DHT) to destroy serotonin (5-HT)-containing nerve terminals throughout the brain. When the animals were killed 3 or 21 days later, we observed a marked decrease in 5-HT content in septum and hippocampus and a parallel decline in *in vitro* high affinity 5-HT uptake. 5-Hydroxyindoleacetic acid (5-HIAA) concentrations also were reduced but by a much smaller extent, resulting in significant increases in the ratio of 5-HIAA to 5-HT. These changes were accompanied by similar increases in the ratio of tryptophan hydroxylase (TPH) activity to 5-HT content. The relative increases in TPH activity resulted from two temporally distinct processes, the first of which appeared to be an activation that could be mimicked *in vitro* by Ca^{2+}-dependent phosphorylation. We conclude that, after partial damage to 5-HT neurons, there is a compensatory increase in the synthesis and release of 5-HT from those terminals that remain.

Subtotal destruction of catecholamine-containing neurons in rat brain produced by 6-hydroxydopamine results in an apparent increase in the synthesis and release of catecholamines within those terminals spared by the neurotoxin [1–5]. This increased turnover is accompanied by an elevation in tyrosine hydroxylase activity relative to the number of remaining terminals [2, 5]. The relative increase in tyrosine hydroxylase activity seems to result from two processes, an initial activation of the enzyme followed by an increase in the number of enzyme molecules per residual terminal. We have proposed that these changes may help to compensate for the injury [6].

There is contradictory evidence concerning the effects of partial damage to serotonin (5-HT)-containing pathways in rat brain. Some investigators have found evidence for increased 5-HT turnover [7] and increased tryptophan hydroxylase (TPH) activity [8] within residual serotonergic terminals after such lesions, but others have not [9–11]. We therefore sought to determine whether partial destruction of central serotonergic projections by the neurotoxin 5,7-dihydroxytryptamine is accompanied by an elevation in 5-HT turnover and, if so, whether this is associated with changes in TPH of the sort we have observed previously for tyrosine hydroxylase after injury to catecholaminergic neurons.

MATERIALS AND METHODS

Materials. Reagents and drugs obtained as follows: 5,7-dihydroxytryptamine creatinine sulfate (5,7-DHT), probenecid, dithiothreitol, L-cysteine, pargyline hydrochloride, 5-hydroxy-L-tryptophan (5-HTP), adenosine 5′-triphosphate disodium salt (ATP), adenosine 3,5-cyclic monophosphate (cAMP), and bovine serum albumin fraction V, from the Sigma Chemical Co., St. Louis, MO; 6-methyl-5,6,7,8-tetrahydropterin-HCl (6MPH$_4$) and *O*-phthalaldehyde from the Calbiochem-Behring Corp., La Jolla, CA; L-(-)-tryptophan from the Aldrich Chemical Co., Milwaukee, WI; nomifensine from Hoechst Russel Pharmaceutical Inc., Somerville, NJ; desmethylimipramine HCl from the Merrell Dow Research Center, Cincinnati, OH; and 5-[1,2-^{3}H(N)]hydroxytryptamine, creatinine sulfate from New England Nuclear, Boston, MA. Scintiverse and all remaining reagents were purchased from the Fisher Scientific Co., Pittsburgh, PA, and were of the highest obtainable purity. In addition, Sephadex G-25 medium was obtained from Pharmacia Fine Chemicals, Piscataway, NJ, and Metricel membrane filters were obtained from Gelman Sciences Inc., Ann Arbor, MI.

Animals. Male, Sprague–Dawley rats (Zivic Miller Laboratories, Allison Park, PA) weighing 200–250 g at the outset of each experiment were allowed Purina Rodent Laboratory Chow (Ralston Purina, St. Louis, MO) and tap water *ad lib*. Animals were housed separately in hanging wire cages in a temperature-controlled room (23–25°) in which illumination was provided by fluorescent lighting from 8:00 a.m. to 8:00 p.m. At least 5 days after arrival, animals were anesthetized with ether and then were given intracerebroventricular injections of 20 μl containing 5,7-DHT (120 μg, free base) or vehicle solution (0.1% ascorbic acid in 0.9% NaCl [12]. Nomifensine (15 mg/kg, i.p.), an inhibitor of high-affinity catecholamine uptake [13], was injected 60 min prior to administration of 5,7-DHT to prevent damage to

* Direct correspondence to: Dr. Michael J. Zigmond, Department of Biological Sciences, 573 Crawford Hall, University of Pittsburgh, Pittsburgh, PA 15260.

catecholaminergic neurons and thereby limit the effects of the neurotoxin to serotonergic nerve terminals [14].

All rats were killed by decapitation between 11:00 a.m. and 1:00 p.m., either 3 days or 3 weeks later. Brains were quickly removed and dissected on ice. Hippocampus and septum were taken to assess the effects of 5,7-DHT treatment on representative terminal fields of the raphe nuclei. A midbrain area between the rostral edge of the anterior colliculi and the caudal edge of the posterior colliculi also was removed for comparable investigation of tissue containing a relatively high concentration of 5-HT cell bodies. Brain sections were frozen on dry ice and stored at −70° for 1–3 days prior to estimation of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), the major metabolite of 5-HT, and the activity of TPH, the rate-limiting enzyme in the biosynthesis of 5-HT. High affinity 5-HT uptake was measured in freshly dissected unfrozen tissue.

TPH activity. Hippocampus (pooled from two or three brains), septum (pooled from three or four brains) and midbrain were homogenized on ice in 50 mM Tris acetate buffer, pH 7.6, containing 1 mM dithiothreitol to reduce degradation of 5-HT and denaturation of TPH, and 30 mM NaF to inhibit phosphatase activity. Homogenate was centrifuged at 4° for 30 min at 40,000 *g*. TPH activity in the supernatant fraction was assayed spectrophotofluorometrically using the procedure of Gal and Patterson [15], modified slightly. The incubation mixture contained 50 mM Tris acetate (pH 7.6), 1 mM dithiothreitol, 1 mM pargyline, 30 μg catalase (1017 units), 0.25 mM tryptophan (unless otherwise noted), 0.25 mM $6MPH_4$ and 200 μl of supernatant fractions (0.5 to 2.0 mg protein) in a final reaction volume of 1 ml. These relatively high concentrations of substrate and cofactor were required due to limitations in the sensitivity of the assay. (Preliminary studies had established that the presence of rat liver dihydrobiopterin reductase and NADH did not enhance TPH activity and therefore they were omitted.) Tissue blanks, to which $6MPH_4$ was not added, were prepared separately for each sample. Both external standards containing 5-HTP and reagent blanks were run through the entire assay procedure. Samples were incubated at 37° for 30 min, and the reaction was terminated by boiling. Next, *O*-phthaldehyde was added to a final concentration of 0.17 mM and boiling was continued for 15 min. The amount of 5-hydroxyindoles was estimated by measuring sample fluorescence (excitation 355 nm; emission 470 mm) and subtracting tissue blank fluorescence. The assay was linear with time to at least 30 min and with protein to 2.5 mg/μl in all experimental situations.

Tissue blanks showed relatively high fluorescence, apparently due to endogenous 5-hydroxyindoles. Consequently, while samples from intact animals were well above blank, samples from 5,7-DHT-lesioned animals typically were only 20–60% above blank values. Thus, it was important to run separate tissue blanks for each sample. The presence of 5-HTP was confirmed by examining the excitation–emission specta of tissue samples obtained from control and lesioned animals and comparing them to authentic 5-HTP standard. Samples usually were assayed in triplicate. TPH activity was expressed as pmoles of 5-HTP formed per mg of protein per min.

In some experiments the effects of calcium-dependent phosphorylating conditions on TPH activity were examined. In those studies assays were conducted in the presence of 0.1 mM $CaCl_2$, 0.5 mM ATP, and 8 mM $MgCl_2$. Exogenous protein kinase and calmodulin were not added to the incubation mixture. In other experiments, enzyme kinetics were investigated. The incubation medium contained subsaturating concentrations of $6MPH_4$ (0.32 mM) and oxygen (20%) and one of several concentrations of tryptophan (20–250 μM). An apparent maximal velocity (V_{max}) and apparent affinity constant (K_m), together with standard errors of the mean, were estimated by computer-assisted non-linear, least squares analysis. These studies were conducted only in hippocampus; a similar examination of TPH activity was not done in septum owing to the relatively large amount of tissue required.

Determination of 5-HT and 5-HIAA. 5-HT and 5-HIAA were measured using reverse-phase high performance liquid chromatography (HPLC) according to a minor modification of the method of Mefford and Barchas [16]. In experiments in which TPH activity, 5-HT, and 5-HIAA were measured in the same sample, brain tissue was homogenized at 4° in the Tris acetate buffer described above for the TPH assay and 30 μl of homogenate then was mixed with 170 μl of 0.2 M $HClO_2$ containing 0.4 mM $NaHSO_3$. When TPH activity was not being measured, samples were homogenized directly in 0.1 N $HClO_4$ with $NaHSO_4$. Homogenate was centrifuged for 15 min at 40,000 *g*, and the supernatant fraction was stored at −70° for up to 5 days before being analyzed.

The HPLC system was equipped with a μBondapak C18 column (3.9 × 300 mm, Waters Associates, Milford, MA), a model LC-3 amperometric controller (Bioanalytic Systems, Inc., West Lafayette, IN), and a glassy carbon electrode set at +0.85 V versus Ag/AgCl. The mobile phase consisted of 0.1 M citric acid–sodium acetate buffer (pH 4.1), 0.2 mM EDTA, and 2–5% methanol. Quantitative determinations were made by comparing peak heights of samples with those produced by known concentrations of 5-HT and 5-HIAA. The amounts of 5-HT and 5-HIAA were expressed as μg per g of wet tissue or ng per mg of perchloric acid insoluble protein found in the 40,000 *g* pellet.

Synaptosomal 5-HT uptake. The hippocampus was removed bilaterally. One side was homogenized in 0.1 N $HClO_4$ and used to measure the 5-HT and 5-HIAA content. From the other side a crude synaptosomal fraction was prepared according to the method of Gray and Whittaker [17]. Tissue was homogenized on ice in 20 vol. of unbuffered 0.32 M sucrose using a glass–Teflon homogenizer (clearance, 0.025 cm). Homogenate was centrifuged for 10 min at 1,000 *g* and the supernatant fraction centrifuged for 20 min at 15,000 *g*. The resulting pellet (P_2), containing synaptosomes as well as free mitochondria and membrane fragments, was resuspended gently in 5 vol. of 0.32 M sucrose. Fifty microliters of this P_2 fraction containing approximately 0.25 mg

protein was preincubated for 5 min at 37° in Krebs–Ringer phosphate medium containing 122 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 1.25 mM $CaCl_2$, 1 mM ascorbic acid, 10 mM glucose, 20 mM sodium phosphate buffer (pH 7.4) and 20 μM pargyline. Twenty microliters of [^{3}H]5-HT (26.5 Ci/mmole) was added, yielding a final 5-HT concentration of 10 nM, and the incubation was continued. Five minutes later, a 380-μl aliquot was removed from the incubation mixture and applied to a premoistened filter (0.45 μm pore size). Filters were washed twice with 2.0 ml of ice-cold incubation buffer containing 5 mM 5-HT, dried, placed into scintillation vials, and eluted with 1 ml of 0.2 M $HClO_4$. Samples were quantified by liquid scintillation spectroscopy after addition of 10 ml Scintiverse. Saturable high-affinity uptake was defined as the difference between total tissue retention of tritium and the uptake obtained in the presence of 10 μM unlabeled 5-HT (nonsaturable uptake). This uptake was temperature dependent and inhibited by 0.1 nM desmethylimipramine, but it was not affected by either 0.2 μM dopamine or 0.2 μM norepinephrine. All samples and blanks were run in triplicate. Saturable uptake was found to be proportional to incubation time for 5 min and to protein concentration at least within a range of 0.02 to 0.4 mg/sample. Uptake velocity was expressed as pmoles [^{3}H]5-HT per mg of soluble protein per min. Approximately 20,000 and 4,500 gross cpm usually were found in samples from control and 5,7-DHT lesioned rats respectively. Nonsaturable uptake yielded about 2,500 cpm in both groups of rats.

Protein. Soluble protein content in the supernatant fraction used for TPH assay and in the synaptosomal fraction was determined according to the method of Bradford [18]. Tissue samples containing approximately 10 μg of protein in 150 μl of 10 mM Tris acetate buffer were mixed with 1.5 ml of reagent containing 0.01% Coomassie Brilliant Blue G-250, 4.7% ethanol, and 8.5% H_3PO_4. After a period of 20 min, samples and standards containing bovine serum albumin were measured spectrophotometrically at 595 nm. When 5-HT and 5-HIAA were assayed, $HClO_4$-insoluble protein was estimated according to the method of Lowry *et al.* [19] after sonication of the 40,000 *g* pellet in 1 N NaOH. Both assays were linear for up to 15 μg of protein. (The ratio of soluble to insoluble protein content as determined by these methods is approximately 1:6.)

RESULTS

In vitro *5-HT uptake and 5-HT content.* To evaluate the extent to which serotonergic terminals were destroyed by 5,7-DHT treatment, we examined *in vitro* [^{3}H]5-HT uptake and 5-HT content in hippocampus. Three days after 120 μg of 5,7-DHT had been administered intraventricularly, [^{3}H]5-HT uptake by crude synaptosomal fractions was reduced to 7% of the control rate. This was accompanied by a comparable decrease in 5-HT content. Similar results were obtained 21 days after 5,7-DHT administration (Fig. 1). These results are consistent with previous observations suggesting that 5,7-DHT produces a long-term degeneration of serotonergic nerve terminals [20].

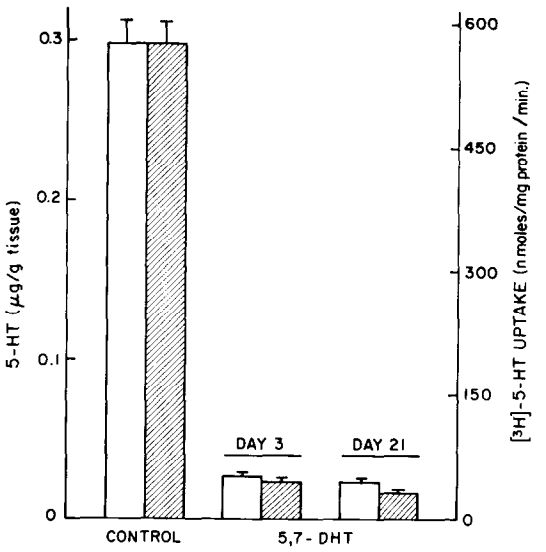

Fig. 1. Effect of 5,7-dihydroxytryptamine (5,7-DHT) on hippocampal serotonin (5-HT) content (open bars) and high-affinity uptake (hatched bar). Animals were killed 3 or 21 days after surgery (N = 6,6). 5-HT content and high-affinity uptake were measured in separate hippocampi taken from the same animals. Control data (N = 8) obtained at days 3 and 21 were similar to one another and were pooled. Bars represent means ± S.E.M.

5-HT, 5-HIAA and TPH activity. Intraventricular injection of 5,7-DHT decreased hippocampal 5-HIAA concentration to a significantly smaller extent than 5-HT concentration. TPH activity also was decreased to a smaller extent than 5-HT content (Figs. 2A and 3). The disparity among these three measures was even greater in the septum, another terminal-rich area (Fig. 2B). These results suggest that TPH activity and 5-HT turnover both were increased within terminals spared by the lesion.

Changes in 5-HT, 5-HIAA and TPH after 5,7-DHT administration also were investigated in the midbrain area, which contains the raphe nuclei. Relatively small reductions in 5-HT content were observed 3 and 21 days post-lesion, suggesting a greater toxic effect of 5,7-DHT on terminals than on cell bodies. In contrast, although no decrease in midbrain 5-HIAA was observed at 3 days, at 21 days the 5-HIAA content of midbrain was reduced to a greater extent than 5-HT (Figs. 2C and 3). These apparent fluctuations in 5-HT turnover were accompanied by parallel alterations in TPH activity (Figs. 2C and 3), and suggest that an initial increase in TPH activity and 5-HT turnover was followed by a decrease in both variables to below control levels.

Kinetic analysis of TPH activity. To further examine the relative increase in TPH activity in terminal-rich areas, we measured the enzyme in hippocampus as a function of tryptophan concentration. Three days after 5,7-DHT administration, the ratio of the apparent V_{max} for TPH to residual 5-HT content had increased to 144% of control, whereas the apparent K_m for tryptophan decreased to 56% of control. By 21 days, V_{max}/5-HT had further increased to 219%

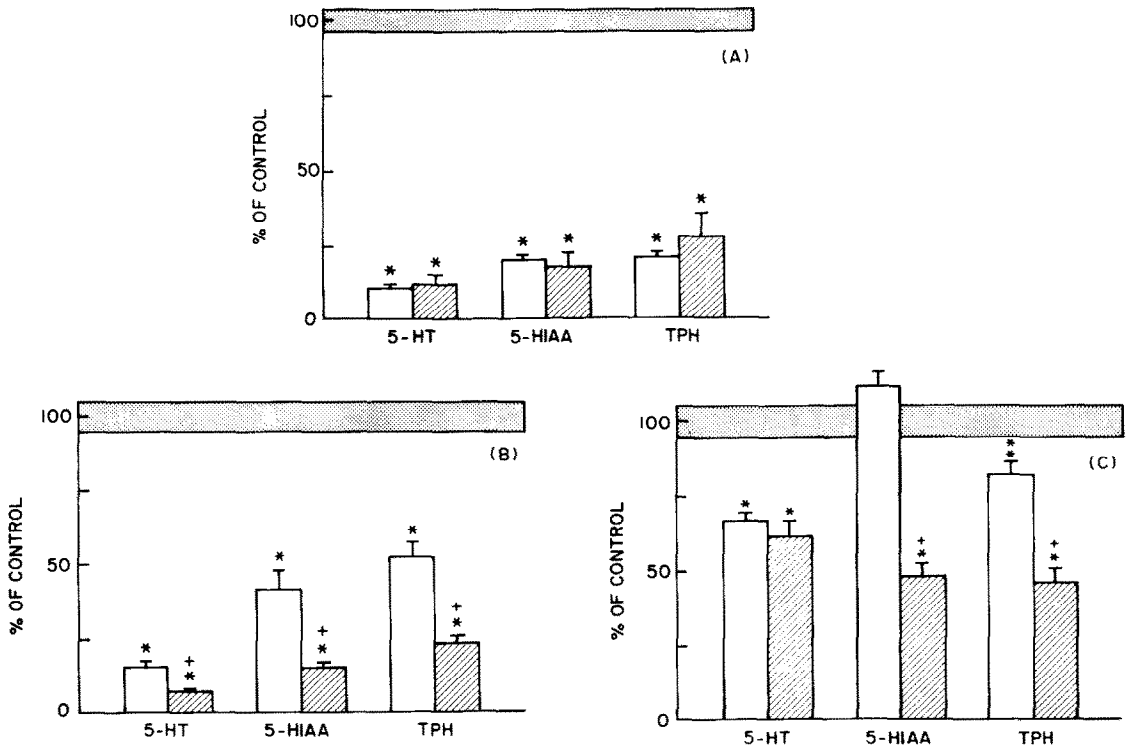

Fig. 2. Effect of 5,7-dihydroxytryptamine (5,7-DHT) on serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), and tryptophan hydroxylase (TPH) activity in different areas of the rat brain, 3 (open bars) and 21 days (hatched bars) post-lesion (N = 12–18 and 6–7, respectively). Control data 3 and 21 days after vehicle injections were similar to one another and were pooled (N = 17–19). Values represent means ± S.E.M. (A) In hippocampus, control values: 5-HT, 3.5 ± 0.1 ng/mg protein; 5-HIAA, 3.1 ± 0.1 ng/mg protein; TPH, 25.2 ± 0.8 pmoles/mg protein/min. (B) In septum, control values: 5-HT, 9.2 ± 0.3 ng/mg protein; 5-HIAA, 4.8 ± 0.2 ng/mg protein; TPH, 66 ± 2 pmoles/mg protein/min. (C) In midbrain, control values: 5-HT, 7.9 ± 0.4 ng/mg protein; 5-HIAA, 7.5 ± 0.4 ng/mg protein; TPH, 225 ± 10 pmoles/mg protein/min. Asterisks represent statistically significant differences between control and 5,7-DHT-lesioned rats (* $P < 0.001$, ** $P < 0.01$); crosses represent statistically significant differences between 3 and 21 days post-lesion ($P < 0.001$).

of control, while K_m had returned to normal (Fig. 4).

A relative increase in TPH activity may reflect activation of existing TPH or increased concentration of the enzyme. Numerous observations indicate that the activation of TPH can occur through a Ca^{2+}-dependent phosphorylation process [21–25]. Consistent with such an hypothesis, we found that incubation of whole brain (excluding cerebellum) from control rats under Ca^{2+}-dependent phosphorylating conditions resulted in a large rise in TPH activity that was characterized by an increase in V_{max} to 180% of baseline and a decrease of apparent K_m for tryptophan to 54% of baseline (Fig. 5). A much smaller activation of tryptophan hydroxylase was obtained if calcium was deleted from the phosphorylation mix (data not shown).

The changes in TPH activity produced *in vitro* by incubation under phosphorylating conditions were similar to the changes produced in hippocampus 3 days after 5,7-DHT administration. To evaluate whether this similarity reflected a common underlying mechanism, septum and hippocampus were obtained 3 and 21 days post-lesion and incubated under Ca^{2+}-dependent phosphorylating conditions. Twenty-one days after 5,7-DHT administration, Ca^{2+}-dependent phosphorylating conditions uniformly increased TPH activity to the same extent as in control animals. In contrast, at 3 days a considerable degree of variability was observed in the effects of phosphorylating conditions (Table 1). An examination of the results from individual samples indicated that, at 3 days, the effects of phosphorylating conditions were inversely related to the ratio of basal TPH activity to 5-HT content, i.e. samples with higher initial values of TPH/5-HT had a smaller response to Ca^{2+} (Fig. 6).

DISCUSSION

Intraventricular injection of 5,7-DHT resulted in a long-lasting decrease of 5-HT content in the terminal-rich areas of hippocampus and septum, as expected from previous studies [8, 20]. In hippocampus, 5-HT depletion was accompanied by a parallel decrease in *in vitro* high-affinity 5-HT uptake. When measured in the presence of sufficiently low concentrations of [^{3}H]5-HT, as in the present experiments, 5-HT uptake appears to be a specific characteristic of serotonergic terminals. It

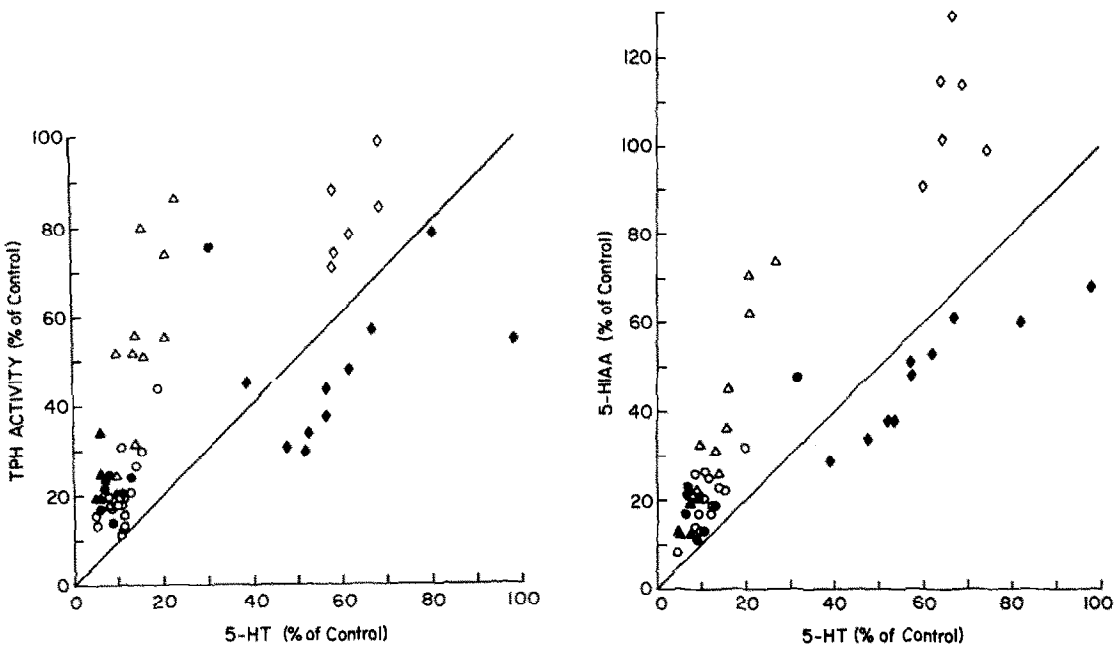

Fig. 3. Tryptophan hydroxylase (TPH) activity and 5-hydroxyindoleacetic acid (5-HIAA) content as a function of serotonin (5-HT) loss. Nomifensine-pretreated rats were injected intracerebroventricularly with 120 μg of 5,7-dihydroxytryptamine or vehicle. Animals were killed 3 days (open symbols) or 21 days (filled symbols) after surgery. Serotonin and 5-HIAA contents and TPH activity were measured in hippocampus (○, ●), septum (△, ▲), and midbrain (◇, ◆). Symbols represent data from individual animals; lines represent the hypothetical case of equal losses of 5-HT and either 5-HIAA or TPH, and are included to facilitate comparison with the data. Note that values for hippocampus and midbrain fall above the lines at both 3 and 21 days indicating the presence of more TPH activity and 5-HIAA than would be predicted by the loss of 5-HT. In contrast, the values for midbrain fall slightly above the lines at 3 days and below the lines at 21 days.

Table 1. Effect of calcium-dependent phosphorylating conditions on tryptophan hydroxylase (TPH) activity in sham and 5,7-dihydroxytryptamine (5,7-DHT) lesioned rats

	N	5-HT (ng/mg protein)	TPH activity (pmoles/mg proteins/min)		TPH activation (Phosphorylating / Basal)
			Basal conditions	Phosphorylating conditions	
Hippocampus					
Sham	9	3.50 ± 0.62	25.5 ± 1.2	53.8 ± 8.1*	2.10 ± 0.07
5,7-DHT (3 days)	17	0.32 ± 0.02	4.1 ± 0.3	6.4 ± 0.5*	1.59 ± 0.11†
5,7-DHT (21 days)	7	0.42 ± 0.11	6.9 ± 2.0	14.3 ± 3.9*	2.09 ± 0.15
Septum					
Sham	14	9.02 ± 0.48	67.1 ± 1.8	127.2 ± 6.4*	1.88 ± 0.06
5,7-DHT (3 days)	9	1.33 ± 0.19	43.2 ± 7.3	62.6 ± 9.7*	1.59 ± 0.15
5,7-DHT (21 days)	6	0.66 ± 0.16	15.6 ± 1.6	31.7 ± 4.4*	2.01 ± 0.09
Midbrain					
Sham	5	8.46 ± 0.57	244.5 ± 12.1	394.0 ± 21.5*	1.62 ± 0.07
5,7-DHT (3 days)	6	5.30 ± 0.19	185.3 ± 9.5	298.5 ± 13.5*	1.63 ± 0.09

Tissue supernatant fraction was incubated under basal conditions and in the presence of 0.1 mM $CaCl_2$, 0.5 mM ATP, 8 mM $MgCl_2$ (phosphorylating conditions). "TPH activation" was defined as the ratio of TPH activity under phosphorylating conditions to TPH activity under basal conditions for each tissue sample. Values represent means of N experiments ± standard error of the mean.

* $P < 0.001$ (difference between TPH activity in control and phosphorylating conditions).

† < 0.001 (difference between sham and brain-lesioned rats).

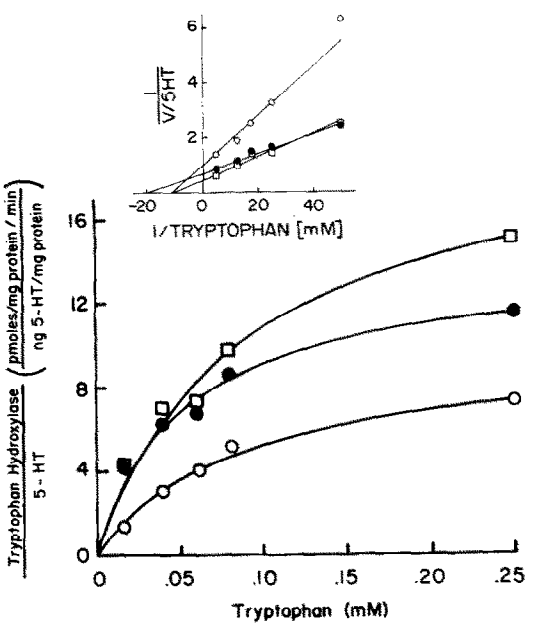

Fig. 4. Kinetic analysis of tryptophan hydroxylase (TPH) activity in hippocampus. TPH activity was measured as a function of tryptophan concentration in control hippocampus (○), and in tissue from animals given 5,7-dihydroxytryptamine 3 days (●) and 21 days (□) previously. Curves were determined by non-linear least squares analysis. The inset shows a Lineweaver–Burk plot of the data. The kinetic constants (± S.E.M.) as determined by nonlinear analysis were as follows: controls: K_m, 90 ± 15 μM; V_{max}, 10.0 ± 0.9 pmoles/ng 5-HT/min. Three-days post-lesion: K_m, 51 ± 10 μM; V_{max}, 14.5 ± 1.5 pmoles/ng 5-HT/min. Twenty-one days post-lesion: K_m, 109 ± 17 μM; V_{max}, 22.0 ± 2.0 pmoles/ng 5-HT/min. The serotonin values (in ng/mg protein) for these groups were 3.52 ± 0.31, 0.49 ± 0.24, and 0.26 ± 0.03, respectively. Values represent means of four to six experiments, in each of which six to eight animals were used.

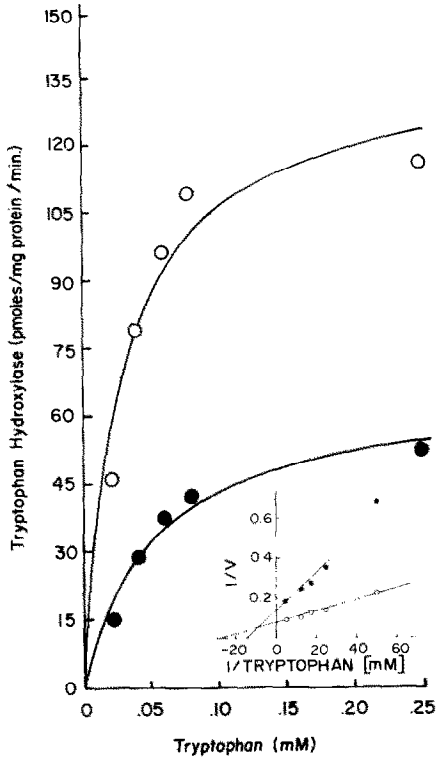

Fig. 5. Kinetic analysis of the effects of phosphorylating conditions on tryptophan hydroxylase (TPH) activity. TPH activity was measured in supernatant fraction prepared from whole rat brain (excluding cerebellum) in the absence (control condition, ○) and in the presence of 0.1 mM $CaCl_2$, 0.5 mM ATP, and 8 mM $MgCl_2$ (phosphorylating condition, ●). Curves were determined by non-linear least squares analysis. The insert shows a Lineweaver–Burk plot of the data. The kinetic constants (± S.E.M.) determined by nonlinear analysis were as follows: control: K_m, 70 ± 8 μM; V_{max}, 74 ± 4 pmoles/mg protein/min. Phosphorylating conditions: K_m, 38 ± 7 μM; V_{max}, 132 ± 11 pmoles/mg protein/min. Values represent means of four experiments, in each of which six to eight animals were used.

correlates well with 5-HT content in different brain areas [26], it decreases after lesion of 5-HT-containing neurons [20, 26, 27], and we have found that it is not inhibited by other monoamines (unpublished observations). The parallel decrease in specific high-affinity 5-HT uptake and 5-HT content suggests that either measure can serve as an index of the extent of terminal degeneration.

The levels of 5-HIAA also were reduced in hippocampus and septum after the lesions. However, the magnitude of this decrease was much less than the decrease in 5-HT content and, consequently, the ratio of 5-HIAA to 5-HT was increased by 40–70% in hippocampus and by more than 100% in septum. Because 5-HIAA is the major metabolite of 5-HT in brain, an increase in 5-HIAA/5-HT may indicate enhanced turnover of 5-HT in residual terminals. This possibility is supported by a previous report that formation of [^{3}H]5-HIAA from [^{3}H]tryptophan in medulla and spinal cord increases after 5,6-DHT administration [7]. Because increases in 5-HIAA also occur during electrical stimulation of serotonergic neurons [28, 29], these results may reflect an increase in 5-HT release from nerve terminals spared by the neurotoxin.

Several previous reports have failed to detect an increase in turnover of 5-HT in the brain after treatment with 5,6- or 5,7-DHT [9–11]. There are at least two possible explanations for this discrepancy. First previous studies have involved analyses of whole brain. Thus, the regional heterogeneity of the changes may have obscured the clear increases in 5-HT turnover that we have observed when selected tissues were examined individually. Second, previous investigators appear to have studied the effects of smaller brain lesions than those reported here. Although we have not examined 5-HT turnover as a function of 5-HT depletion, analogous studies of damaged catecholaminergic systems suggest that 50–60% destruction may be required before changes in turnover are detected [5].

For an increase in 5-HT turnover to be maintained, it must be accompanied by an increase in the rate of tryptophan hydroxylation, the rate-limiting step in 5-HT synthesis. In fact, we observed that, like the decline in 5-HIAA, the reduction of TPH activity after 5,7-DHT administration was much less than

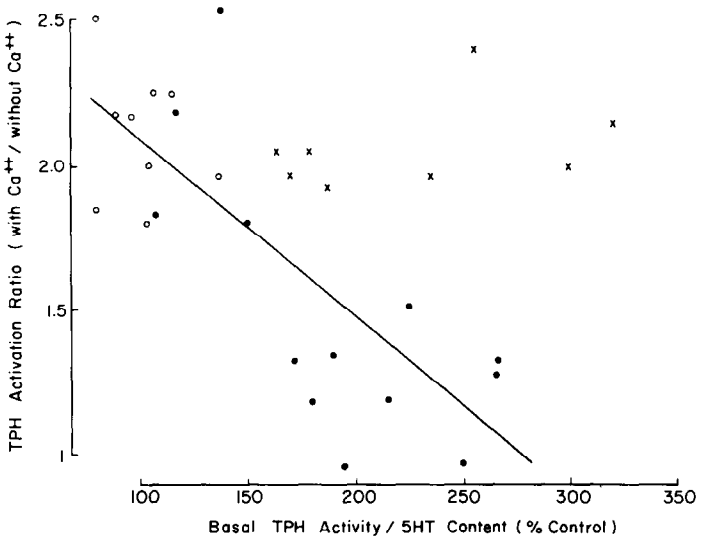

Fig. 6. Effect of incubation under calcium-dependent phosphorylating conditions on tryptophan hydroxylase (TPH) activity. Homogenate was prepared from hippocampus of control animals (○) and from animals that had received 5,7-dihydroxytryptamine (5,7-DHT) either 3 days (●) or 21 days (×) earlier. TPH activity was measured under Ca^{2+}-dependent phosphorylating conditions or basal conditions. The resulting activation ratio was plotted as a function of the ratio of basal TPH activity to endogenous serotonin (5-HT) content. Shown is a linear regression line for the data from control and 3-day samples ($r = -0.79$). The data from 21-day samples were excluded from the analysis. Note that, at 3 days post-lesion, the four samples exhibiting the least change in the ratio of basal TPH activity to 5-HT content (<150% of control) were fully activated by *in vitro* phosphorylating conditions, whereas the nine samples exhibiting the greatest change (>150% of control) showed little or no effect of the *in vitro* incubation. In contrast, all eight of the samples examined 21 days post-lesion were fully activated by *in vitro* phosphorylating conditions. (Comparable results were obtained from septal samples.)

the loss of 5-HT (see also Ref. 8). We interpret these results to indicate that an increase in TPH activity had occurred in residual serotonergic neurons.

Clewans and Azmitia [30] have reported recently that the direct administration of 5,7-DHT into the cingulum bundle also results in an initial reduction in TPH activity in hippocampus, followed by a gradual return toward normal. In contrast to our results with intraventricular 5,7-DHT, however, the rise in TPH activity after intracerebral injections of the toxin appears to be accompanied by a reinnervation of hippocampus [31] and thus may not present an increase in enzyme activity per terminal.

A change in enzyme activity per terminal can result from an activation of existing TPH molecules or from an increase in the availability of new enzyme molecules. There are several reasons to believe that the relative increase in TPH activity at 21 days post-operative probably involves an increase in enzyme protein within the residual terminals. First, this increase was maximal in midbrain at 3 days while in hippocampus and septum enzyme activity continued to increase, suggesting the gradual transport of enzyme from cell body to terminal. Second, at 21 days the relative increase in TPH activity was associated with an increase in apparent V_{max} and no change in the affinity of enzyme for tryptophan. Moreover, the enzyme could be fully activated by Ca^{2+}-dependent phosphorylating conditions. These latter observations suggest that the increase in TPH activity cannot be attributed to the activation of pre-existing molecules (see below).

A relative increase in TPH activity in terminal regions frequently was observed 3 days after 5,7-DHT administration, as well. We believe that this earlier affect may be attributed to an activation of TPH molecules present in residual terminals at the time of injury. Unlike the results obtained at 21 days, an increase in TPH/5-HT was not always present at 3 days post-lesion, a result that is consistent with an easily reversible modification of the TPH molecule. Moreover, the increase in TPH activity at 3 days, when present, was associated with a decrease in the K_m of the enzyme for tryptophan. This effect is similar to the changes in TPH activity that occur after acute 5-HT receptor blockade [32]. Finally, the apparent increase in TPH activity observed 3 days after 5,7-DHT could be mimicked by incubation of control samples under Ca^{2+}-dependent protein phosphorylating conditions; in contrast, these same phosphorylating conditions had little effect in tissue from the 5,7-DHT-lesioned animals.

Harvey and Gal [33] observed that, 10 days after electrolytic lesion of raphe nuclei, 5-HT content in septum and hippocampus had decreased by 70%. TPH activity was reduced by 64% in hippocampus but only by 19% in septum. On the basis of their findings the authors suggested that a large portion of the TPH in septum might be located outside serotonergic neurons. The present report suggests instead that after such lesions there is a gradual increase in TPH enzyme within residual terminals, a process which should occur more slowly in areas further from the raphe nucleus (e.g. hippocampus)

than in less distant areas (e.g. septum). This interpretation also is in agreement with the observation of Victor and coworkers [34] that the increase of TPH activity after intraventricular injection of 5,6-dihydroxytryptamine progresses from caudal to rostral areas within the brain.

Under normal conditions, the firing rate of raphe cells appears to be held under inhibitory control by means of collateral branches of the serotonergic axons [35]. By producing an initial decrease in the amount of 5-HT released into extracellular fluid, 5,7–DHT should reduce such negative feedback and thereby trigger a compensatory increase in serotonergic activity. The activation of TPH may be a consequence of that increase in serotonergic activity. It has been shown that depolarization of brain slices causes a Ca^{2+}-dependent activation of TPH [22, 23, 36], and similar results have been obtained recently using *in vivo* stimulation of serotonergic neurons [37]. The apparent increase in the amount of TPH enzyme may represent a more gradual response to this same reduction in negative feedback.

These changes in residual 5-HT terminals are reminiscent of changes that we have described in central noradrenergic nerve terminals that remain after subtotal terminal destruction with 6-hydroxydopamine. Specifically, we find an increased electrophysiological activity of these neurons [38] that is associated with an initial activation of tyrosine hydroxylase in hippocampus, manifested by an increased affinity of the enzyme for its pteridine cofactor [1]. These changes gradually are replaced by an increase in the apparent V_{max} for tyrosine hydroxylase that probably occurs as a result of increased transport of the enzyme from locus coeruleus [2]. We also have observed similar phenomena in dopaminergic terminals in striatum [5] and in the sympathoadrenal system [39]. Thus, increased synthesis and release of transmitter appear to represent a general compensatory response of monoaminergic systems to partial injury. This increased release, coupled with decreased inactivation due to the loss of reuptake sites, may help to restore the normal influence of monoaminergic neurons over their target cells [6].

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