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# Effect of Recurrent Stress on Postnatal Increase of Tyrosine Hydroxylase

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TORDA, C. *Effect of recurrent stress on postnatal increase of tyrosine hydroxylase.* PHARMAC. BIOCHEM. BEHAV. 3(5) 735–738, 1975. –Tyrosine hydroxylase is present at birth and reaches adult levels in the hypothalamus usually during the second month. Recurrent stimulation of intrahypothalamic noradrenergic structures shortened this period of maturation in a statistically significant manner.

Tyrosine hydroxylase Neonate Recurrent stresses Hypothalamus

INTRACEREBRAL catecholaminergic systems undergo morphological [24,29], biochemical [11, 13, 16, 22, 30] and functional [29,30] changes in a species-dependent time sequence. In spite of morphological immaturity, the newborn brain is able to perform most basic biochemical processes involved in synthesis, storage, release, reuptake and catabolism of norepinephrine [4,26]. Immature noradrenergic neurons fulfill the special function to signal the emergence of homeostatic disquilibria of basic vegetative functions after birth, a function lost during the process of maturation [29,30].

Function of hypothalamic and reticular noradrenergic mechanisms concur with norepinephrine release. Therefore, their performance depends on increased turnover and synthesis of norepinephrine. The initiation and rate-limiting steps in the biosynthesis of norepinephrine depend on tyrosine hydroxylase (L-tyrosine, tetrahydropterin oxygen oxidoreductase (EC 1.10.3.1) [18].

The effects of recurrent postnatal stresses (starvation and electrical stimulation of hypothalamic nuclei) on the speed of maturation of noradrenergic structures have been studied in the present work by observations on tissue levels of tyrosine hydroxlase.

## METHOD

### *Animals and Procedure*

Tyrosine hydroxylase levels of hypothalamus of male albino rats (Sprague-Dawley type) and kittens were studied from birth to adulthood. The animals were divided into 4 groups: (1) controls, (2) stressed, (3) electrode-implanted stressed, and (4) sham-operated controls. The controls (Groups 1 and 4) were raised without interference. The animals in Group 2 were exposed to starvation for half an hour before every feeding. The animals in Group 3 received 6 times daily for 30 min rectangular pulses (1/sec frequency, 40 $\mu$A intensity) by means of hypothalamic microelectrodes (platinum, 1 $\mu$ tip diameter). The electrodes were lowered daily into the hypothalamus through implanted electrode guides fastened to the pierced skull with acrylic dental cement during the second or third postnatal day. The position of the electrode tips were posthumously tested (histologically) in a few animals that were randomly selected at various ages. Implantation was performed under Nembutal anesthesia. Daily insertion of electrodes did not require anesthesia. Group 1 served as control to Group 2, Group 4 to Group 3.

Tyrosine hydroxylase activity was studied following the method of Nagatsu *et al.* [19], as modified by Kuczenski [14].

### *Materials*

L-3,5-$^3$[H]tyrosine (30 Ci/nmole) was obtained from New England Nuclear Co. The tyrosine was further purified by passage through a column of Dowex 50 W-$X_4$ [$H^+$]. The synthetic factor, 2-amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine ($DMPH_4$) was obtained from Calbiochem. Triton X-100 and 3-iodotyrosine were purchased from Sigma Chemical Co. All other chemicals were of maximum purity.

### *Assay*

The animals were sacrificed by decapitation. The brains were removed and were placed in 0.32 M sucrose at $-1^\circ$C. The hypothalamus was dissected on ice. The hypothalami were homogenized in 50 vol. ice-cold 2 mM potassium phosphate buffer, at pH 7.0, using a Thomas glass-Teflon homogenizer with 0.010 cm clearance. An aliquot of the homogenate was diluted into an equal volume of 0.2 percent Tirton X-100 in 2 mM phosphate buffer for measurement of whole homogenate tyrosine hydroxylase activity. Tyrosine hydroxylase activity was assayed by the method of Nagatsu *et al.* [19]. The standard incubation mixture contained 3 $\mu$M 3,5-[$^3$H] tyrosine (specific activity 1 mCi/$\mu$mole), 1.0 mM $DMPH_4$, 50 mM 2-mercaptoethanol,

TABLE 1

EFFECT OF RECURRENT POSTNATAL STRESS ON TYROSINE HYDROXYLASE ACTIVITY OF HYPOTHALAMUS

| | RAT | | | | CAT | | | |
|---|---|---|---|---|---|---|---|---|
| | Control* | | Recurrent Stress† | | Control* | | Recurrent Stress† | |
| Postnatal Days | (nmol/g)/h | Percent of Adult | (nmol/g)/h | Percent of Normal Adult | (nmol/g)/h | Percent of Adult | (nmol/g)/h | Percent of Normal Adult |
| 0 | 0.27 ± 0.020‡ | 6 | 0.27 ± 0.020 | 6 | 0.21 ± 0.013 | 5 | 0.21 ± 0.015 | 5 |
| 7 | 0.45 ± 0.025 | 10 | 0.45 ± 0.026 | 10 | 0.41 ± 0.032 | 10 | 0.41 ± 0.030 | 10 |
| 10 | 0.90 ± 0.031 | 20 | 1.29 ± 0.038 | 29 | 0.82 ± 0.042 | 20 | 1.27 ± 0.050 | 31 |
| 15 | 1.60 ± 0.046 | 36 | 2.59 ± 0.057§ | 58 | 1.47 ± 0.065 | 36 | 2.34 ± 0.122§ | 57 |
| 20 | 2.09 ± 0.082 | 47 | 3.48 ± 0.112§ | 78 | 1.92 ± 0.084 | 47 | 3.12 ± 0.157§ | 76 |
| 30 | 2.86 ± 0.099 | 64 | 4.38 ± 0.101§ | 98 | 2.62 ± 0.097 | 64 | 4.02 ± 0.161§ | 98 |
| 40 | 3.57 ± 0.040 | 80 | 5.43 ± 0.174§ | 121 | 3.28 ± 0.107 | 80 | 5.13 ± 0.125§ | 125 |
| Adult | 4.46 ± 0.023 | 100 | 6.19 ± 0.198§ | 136 | 4.10 ± 0.180 | 100 | 5.74 ± 0.136§ | 140 |

*Control group contains values obtained from both intact and sham-operated animals (Groups 1 and 4) because of comparable values.

†Similar results were obtained from starved animals and animals stimulated by rectangular pulses.

‡Every value represents the average of 12 values obtained from different stressed animals, followed by the S.E. of mean, and 24 values obtained from different control animals (12 from Group 1, 12 from Group 4).

§$p<0.005$. These are statistically significant changes.

0.436 mM $FeSO_4$, and 0.11 M Tris-acetate buffer to give a final pH of 5.8 (at 37°C). Typical incubations were for 20 min. Blanks consisted of active enzyme incubated for an identical time period in the presence of $3 \times 10^{-4}$ M 3-iodotyrosine. Radioactivity was ascertained in a Beckman LS-250 liquid scintillation spectrometer with Aquasol (New England Nuclear Co.) as the scintillation fluid.

## RESULTS

The tyrosine hydroxylase levels of hypothalami of the various animals are summarized in Table 1. Tyrosine hydroxylase activity did exist already at birth, and seemed to reach adult levels during the second half of the second month in both the rat and the cat brains of controls. Adult levels were reached somewhat earlier in the animals exposed to recurrent and prolonged stress. The differences between the tyrosine hydroxylase levels of controls and animals exposed to stresses were statistically significant. With the method used significant differences could not be detected in the tyrosine hydroxylase levels of animals exposed to hunger and animals exposed to electrical stimulation of the hypothalamus.

## DISCUSSION

Tissue levels and enzymatic activity of tyrosine hydroxylase seem to parellel the function of catecholaminergic systems [21,23]. Traces of tyrosine hydroxylase precede detectable traces of catecholamines during the gestation period [2,16]. During the early postnatal period the increasing levels of tyrosine hydroxylase reflect the development of axon terminals and synapses [11, 21, 25, 26]. Thereafter, the catecholamine requirements of tissues seem in some way to relate to the amount of enzymatic activity of tyrosine hydroxylase (e.g. changes in activity of catecholaminergic neurons [1,10], adrenergic transsynaptic activity [3, 9, 17, 28], electroshock [6], different types of stresses (including stimulation of hypothalamic nuclei by electrical pulses (Table 1), hunger (Table 1), cold [4, 5, 7, 8, 27], reserpine [12,23], etc.).

Brain tyrosine hydroxylase occurs *in vivo* in a partially inhibited form, and enzymatic activity may temporarily increase due to changes of local factors [2, 13, 15, 21, 32], including decreased concentrations of tissue catecholamines. This increase occurs with a short latency. Increase of enzymatic activity due to increased tissue content of the enzyme usually requires a longer latency [12, 17, 20, 34], e.g. Otten *et al.* [20] observed that 2 hr exposure of adult animals to stress initiates a 24 hr increase of tyrosine hydroxylase protein at ribosomal translation level. Preliminary results of protein determinations ([31] by method of [34]) suggest that recurrent postnatal stressful situations studied in the here presented work increase tyrosine hydroxylase activity by both mechanisms: temporary enzyme activation and increase of enzyme proteins. Premature achievement of adult tissue enzyme levels (Table 1) and permanence of increased tyrosine hydroxylase levels resulting from continued recurrence of stressful situations in later life [30] seem to depend on increase of enzyme proteins [31].

## REFERENCES


1. Alousi, A., and N. Weiner. The regulation of norepinephrine synthesis in sympathetic nerves: Effect of nerve stimulation, cocaine and catecholamine releasing agents. *Proc. natn. Acad. Sci., U.S.A.*, **56**: 1491–1496, 1966.
2. Bhagat, B. D. *Recent Advances in Adrenergic Mechanisms.* Springfield: C. Thomas, 1971.
3. Black, I. B., and S. C. Green. Transsynaptic regulation of adrenergic neuron development: Inhibition by ganglionic blockade. *J. Neurochem.* **23**: 7–15, 1973.
4. Carlsson, A. The role of catecholamines in cold adaptation. *Pharmac. Rev.* **18**: 291–301, 1966.
5. Dairman, W., And S. Udenfriend. Effects of ganglionic blocking agents on the increased synthesis of catecholamines resulting from α-blocking on exposure to cold. *Biochem. Pharmac.* **19**: 979–984, 1970.
6. Glowinski, J., J. M. Musacchio, L. Julan, and S. Kety. Increase in rat brain tyrosine hydroxylase activity produced by electroconvulsive shock. *Proc. natn. Acad. Sci. U.S.A.* **63**: 1117–1119, 1969.
7. Gordon, R., S. Spector, A. Sjoerdsma, and S. Udenfriend. Increased synthesis of norepinephrine and epinphrine in the intact rat during exercise and exposure to cold. *J. Pharmac. exp. Ther.* **153**: 440–447, 1966.
8. Guidotti, A., Z. Zivkovic, R. Pfeiffer, and E. Costa. Involvement of 3′,5′-monophosphate in the increase of tyrosine hydroxylase activity elicited by cold exposure. *Archs Pharmac.* **278**: 195–206, 1973.
9. Hanbauer, I., I. J. Kopin, and E. Costa. Mechanisms involved in the trans-synaptic increase of tyrosine hydroxylase and dopamine-β-hydroxylase activity in sympathetic ganglia. *Archs Pharmac.* **280**: 39–48, 1973.
10. Hendry, I. A., L. L. Iversen and I. B. Black. A comparison of the neural regulation of tyrosine hydroxylase activity in sympathetic ganglia of adult mice and rats. *J. Neurochem.* **20**: 1683–1689, 1973.
11. Iversen, L. L., J. DeChamplain, J. Glowinski and J. Axelrod. Uptake, storage and metabolism of norepinephrine in tissues of developing rat. *J. Pharmac. exper. Ther.*, **157**: 509–516, 1967.
12. Joh, T. H., C. Geghman, and D. Reis. Immunochemical demonstration of increased activation of tyroid hydroxylase protein in sympathetic ganglia and adrenal medulla elicited by reserpine. *Proc. natn. Acad. Sci. U.S.A.* **70**: 2767–2771, 1973.
13. Kellogg, C., and P. Lundborg. Inhibition of catecholamine synthesis during ontogenic development. *Brain Res.* **61**: 321–329, 1973.
14. Kuczenski, R. T. Rat brain tyrosine hydroxylase. *J. Biol. Chem.* **248**: 2261–2265, 1973.
15. Kuczenski, R. T. and A. J. Mandell. Allosteric activation of hypothalamic tyrosine hydroxylase by ion and sulphated mucopolysaccharides. *J. Neurochem.* **19**: 131–137, 1972.
16. McGeer, E. G., S. Gibson, J. A. Wada and P. L. McGeer. Distribution of tyrosine hydroxylase activity in adult and developing brain. *Can. J. Biochem.* **45**: 1943–1952, 1967.
17. Mueller, R. A., H. Thoenen, and J. Axelrod. Inhibition of trans-synaptically increased tyrosine hydroxylase activity by cycloheximide and actinomycin D. *Molec. Pharmac.* **5**: 463–469, 1969.
18. Nagatsu, T., M. Lewitt, and S. Udenfriend. Tyrosine hydroxylase, the initial step in norepinephrine biosynthesis. *J. Biol. Chem.* **239**: 2910–2917, 1964.
19. Nagatsu, T., M. Levitt, and S. Udenfriend. A rapid and simple radioassay for tryosine hydroxylase activity. *Analyt. Biochem.* **9**: 122–126, 1964.
20. Otten, U., U. Paravicini, O. Oesch, and H. Thoenen. Tyrosine hydroxylase induction: The requirement for completion of transcription. *Experientia* **29**: 765, 1973.
21. Patrick, R. L., and J. D. Barchas. Regulation of catecholamine synthesis in rat brain synaptosomes. *J. Neurochem.* **23**: 7–15, 1974.

22. Porcher, W., and A. Heller. Regional development of catecholamine biosynthesis in rat brain. *J. Neurochem.* **19:** 1917–1930, 1972.
23. Rutledge, C. O., and N. Weiner. The effect of reserpine upon the synthesis of norepinephrine in the isolated rabbit heart. *J. Pharmac. exp. Ther.* **157:** 290–302, 1967.
24. Scheibel, M. E., T. L. Davies, and A. B. Scheibel. Maturation of reticular dendrites: Loss of spines and development of binding. *Expl. Neurol.* **38:** 301–310, 1973.
25. Segal, D. S., and Kuczenski. Tyrosine hydroxylase activity: Regional and subcellular distribution in brain. *Brain Res.* **68:** 261–266, 1974.
26. Stjarne, L.: Studies of catecholamine uptake and storage and release mechanisms. *Acta physiol. scand.* **62:** Suppl 228 1–97, 1964.
27. Thoenen, H. Induction of tyrosine hydroxylase in peripheral and central adrenergic neurons by cold exposure of rats. *Nature* **228:** 861–862, 1970.
28. Thoenen, H., R. A. Mueller, and J. Axelrod. Trans-synaptic induction of adrenal tyrosine hydroxylase. *J. Pharmac. exp. Ther.* **169:** 249–254, 1969.
29. Torda, C. Functions of immature dendrites of the hypothalamic reticular activating neurons of newborn. *Biophys. J.* **15:** 47, 1975.
30. Torda, C. Observations on subcellular mechanisms of generation of compulsive aggressive or anxious behavior. *Fedn. Proc.* **34:** 875, 1975.
31. Torda, C., unpublished data.
32. Udenfriend, S. Tyrosine hydroxylase. *Pharmac. Rev.* **18:** 43–51, 1966.
33. Vogel, W. H., V. Orfei, and B. Century. Activities of enzymes involved in the formation and destruction of biogenic amines in various areas of human brain. *J. Pharmac. exp. Ther.* **165:** 196–203, 1968.
34. Zigmond, R. E., and A. V. P. Mackay. Dissociation of stimulatory and synthetic processes in the induction of tyrosine hydroxylase. *Nature* **247:** 112–113, 1974.