Biochemical Pharmacology, Vol. 24, pp. 1739–1742. Pergamon Press, 1975. Printed in Great Britain.

EFFECTS OF 5,6-DIHYDROXYTRYPTAMINE ON TYROSINE-HYDROXYLASE ACTIVITY IN CENTRAL CATECHOLAMINERGIC NEURONS OF THE RAT

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(Received 4 July 1975; accepted 10 July 1975)

The destruction of the anterior part of the cat raphé system (including nuclei raphé dorsalis and raphé centralis) induces a significant increase in norepinephrine (NE) turnover in the projections of the locus coeruleus (LC)[1]. Similar lesioning in the rat is followed by a significant increase of cortical and hippocampal concentrations of 3-methoxy-4-hydroxy-phenylethyleneglycol sulfate (MOPEG-SO_4)[2], a major metabolite of NE in the brain. These observations have suggested the possibility of a serotoninergic control of the synthesis of NE in the LC by serotonin (5-HT) containing neurons of the raphé system[1,2]. This mechanism of control could involve a modification of tyrosine-hydroxylase (TH) activity, either in the noradrenergic terminals and/or at enzyme synthesis sites in the cell bodies. To test this hypothesis, we studied the regional changes of cerebral TH activity after a destruction of 5-HT containing terminals resulting from the intracisternal injection of 5,6-dihydroxytryptamine(5,6-HT)[3].

Forty male rats (OFA strain, supplied by IFFA-CREDO) weighing 180-220 g and maintained under previously described conditions[4] were studied. Under slight ether anesthesia, 20 rats received an intracisternal injection of 50 µg of 5,6-HT (as a free base, dissolved in 20 µl of sterile saline containing 0,1 mg/ml of ascorbic acid)[5]. Twenty control animals were similarly injected with 20 µl of the saline-ascorbic acid solvent. At various days after the injections, 4 treated and 4 control rats were killed (by rapid decapitation) at the same hour they were injected (9 a.m.). Their brains were stereotaxically cut and a section removed between the A5 and P5 frontal planes (DE GROOT)[6] and quick-frozen on dry-ice. The frontal cortex and neostriatum were dissected from

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the fragment remaining anterior to the A5 plane. The LC (A6 group)[7] and the mesencephalic dopaminergic groups (A10 and A9)[7] were microdissected from the removed brain section according to a technique previously described[8]. Each structure was individually sonicated (20KHz, 40 W, 20 sec) in 0,002 M potassium phosphate buffer, pH 6.00, containing 0,2 % Triton X 100. TH activity was measured in the 10,000 g supernatant by a modification[8,9] of the method of NAGATSU et al[10].

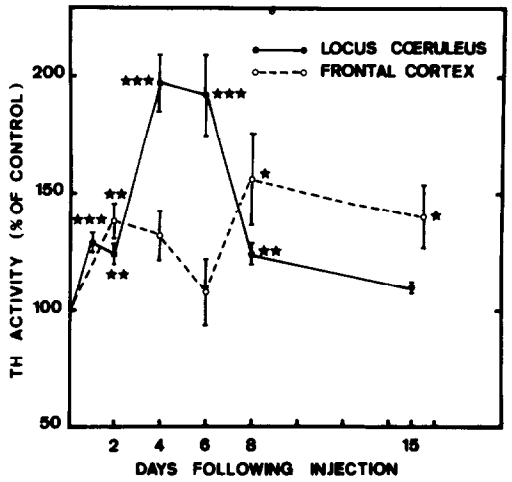

Fig.1 - Effect of intracisternally injected 5,6-HT (50 µg/rat) on tyrosine-hydroxylase (TH) activity in the rat locus coeruleus and frontal cortex at various days following injection. The concentration of tyrosine in each assay was 20 µM and that of $DMPH_4$ 1.14 mM. Each point represents mean ± SEM of 4-8 experimental values expressed as percent of activity in controls. Absolute values of sham injected controls (means + SEM) : 93.51 + 7.46 pM of DOPA/h/structure (locus coeruleus) and 24.71 ± 5.85 pM of DOPA/h/mg protein (frontal cortex). * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

As shown in Fig.1, injection of 5,6-HT was followed by a marked increase in TH activity in the LC ; this increase was significant at 1 day, reached its experimental maximum between 4 (+ 97 %, $p < 0.001$) and 6 days (+ 92 %, $p<0.001$), and returned to the control value between 8 and 15 days. These findings support previous results[5] showing an increased activity in the medulla/pons 4 days after intraventricular administration of similar doses of 5,6-HT. In the frontal cortex, as in the LC, TH activity increased to a first maximum at 2 days (+ 39 %, $p<0.01$). Thereafter, activity decreased to a minimum at 6 days and rose again to a second activity peak at 8 days (+ 56 %, $p < 0.05$) which remained elevated at 15 days (+ 40 %, $p<0.05$).

The elevated TH activity in the LC after 5,6-HT administration could be due to an increased number of enzyme molecules, as previously suggested by other experiments in similar conditions (cofactors in excess), e.g., stress and reserpine administration[11], and unilateral lesioning of the controlateral LC[8]. Furthermore, we observed the same slow pattern of increase of activity (see Fig 1) as in these other experiments.

The two activity peaks measured in the frontal cortex could result from transport of different forms of the enzyme[12] by separate components of the axoplasmic flow[13]. Another explanation is that there would be successive actions of two complementary mechanisms : first, a short lasting activity peak initially induced by a greater functional activity of the noradrenergic terminals (as after stimulation of the LC[14]) and second, a slowly rising and eventually sustained activity peak which reflects the operation of new enzyme originating in the cell bodies. Both mechanisms could exist at the perikaryal level, but would be difficult to differentiate without further kinetic studies.

Dopaminergic terminals have been demonstrated in the frontal cortex[15] and their cell bodies have been found to be localized mainly in the A10 group[16]. Although the effect we observed in this cortical area might also have been due, in part,to changes in TH activity in these neurons (as shown in Fig.2), TH activity in the A10 group did not increase and further, a significant decrease (-13 %, $p < 0.05$) was observed at 15 days. TH activity in the nigro-striatal system first decreased (-14 %, $p < 0.05$ at 2 days and -11 %, $p < 0.05$ at 6 days in the A9 group ; -23 %, $p < 0.02$ at 4 days in the neostriatum) and subsequently increased at 15 days (+24 %, $p < 0.05$ in the A9 group). Thus, no correlation was found between the elevation of TH in the frontal cortex and the changes of TH activity in the A10 group. Moreover, we can confirm the previously reported[17] partial destruction of dopaminergic systems by 5,6-HT. The increased TH activity observed at the 15th day in the nigro-striatal system could be interpreted as an activation of intact dopaminergic cells, as has been found to occur after partial destruction of the A9 group[18].

We hypothesize that elevation of TH activity after 5,6-HT administration occurs in the coeruleo-cortical noradrenergic neurons[7]. This elevation could result from a non-specific drug effect[5], but more likely, the destruction of 5-HT terminals by 5,6-HT, as well as by the coagulation of serotoninergic cell bodies[1,2], suppresses their control of NE synthesis in the noradrenergic neurons of the LC system. Changes in TH activity would represent one of the mechanisms involved in this regulatory process.

This work has been helped through the supports of the INSERM (U52), the CNRS (LA 162), and the DRME (contract 74-232).

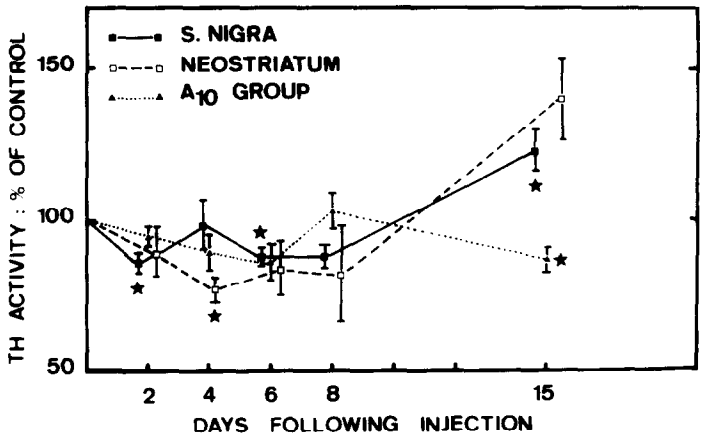

Fig.2 - Effects of intracisternally injected 5,6-HT (50 µg/rat) on TH activity in rat substantia nigra, neostriatum and A10 at various days following injection. Results are expressed as in fig.1. Absolute values of sham injected controls (means ± SEM): 180.81 ± 25.99 (S.Nigra) and 370.37 ± 33.21 (A10 group) pM of DOPA/h/structure, 1052 ± 159 pM of DOPA/h/MG protein (Neostriatum). ★ $p < 0.05$.

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