

CONSEQUENCES OF DECREASED BRAIN SEROTONIN IN THE PYRIDOXINE-DEFICIENT YOUNG RAT

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Abstract

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1. The concentrations of serotonin in various brain areas were significantly decreased in the pyridoxine-deficient young rat.
2. There was no change in the concentration of dopamine.
3. Both B_{max} and K_D of [³H]serotonin binding to membrane preparations from cerebral cortex were increased in deficiency and were restored to normal upon pyridoxine supplementation.
4. There was no change in [³H]spiroperidol binding to corpus striatal membrane preparations in pyridoxine-deficient rats.

Keywords: serotonin, dopamine, receptors, spiroperidol

Introduction

The enzyme aromatic amino acid decarboxylase requires pyridoxal phosphate as a coenzyme. In view of its lack of substrate specificity it has been generally accepted that it is involved in the formation of both the catecholamines and serotonin. Hence, in pyridoxine deficiency parallel decreases in brain of the catecholamines as well as serotonin would be expected. We have reported (Dakshinamurti et al 1976) a significant decrease in brain levels of serotonin with no change in the levels of dopamine and norepinephrine. Various studies including 5-hydroxytryptophan loading indicated that its decarboxylation was decreased in pyridoxine-deficient rat brain. We suggested that the hypothermia and decreased motility seen in the deficient animals might be among the consequences of a functional deficit of the serotonergic system. Sleep studies also indicated a parallel between the effects of pyridoxine deficiency and serotonin deficiency (Kilianma and Fuxe 1977). The durations of deep slow-wave sleep 2 and REM sleep were shortened and in some instances completely abolished in the pyridoxine-deficient rat (Dakshinamurti 1982).

The physiological significance of the reduction of serotonin in various brain regions has been examined further. It is generally recognized that a chronic increase in neurotransmitter concentration desensitizes post-synaptic receptors and conversely, conditions leading to a chronic decrease in neurotransmitter concentration lead to enhanced post-synaptic sensitivity. In this study we have determined the concentrations of dopamine and serotonin and attempted a correlation with receptor sensitivities in various brain regions.

Methods

Animals. Sperm-positive Sprague Dawley female rats were fed a pyridoxine supplemented diet till parturition when they were divided into 2 groups. One group was fed a pyridoxine-deficient diet (Dakshinamurti and Stephens 1969) while the other continued on the supplemented diet. The number of pups with each dam was 8 in the deficient group and 16 in the supplemented

group. The animals were 3 weeks old when used in experiments. In one experiment an additional group consisted of deficient mothers fed a pyridoxine-supplemented diet during only the last week.

Materials. [^3H]-5-hydroxytryptamine (30.3 Ci/mmol) and [^3H]-spiroperidol (34.0 Ci/mmol) were purchased from New England Nuclear Corp. (Boston, MA). Spiroperidol was a gift from Jansen Pharmaceuticals (Beerse, Belgium). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

Experimental procedure. Brain regions were dissected according to Glowinski and Iversen (1966). Tissues were frozen immediately in dry ice and stored at -70°C till used. The method of Hammel et al (1978) was used for serotonin assay and that of Sole and Hussain (1977) for dopamine assay. [^3H]-spiroperidol binding was assayed in striatal membrane preparations essentially according to Creese et al (1978). [^3H]-5-hydroxytryptamine binding was assayed in cerebral cortex membrane preparations using the method of Uzbekov et al (1979). Specific binding data were analyzed according to Scatchard (1949) from which maximal binding (Bmax) and dissociation constant (Kd) were derived by linear regression analysis. Analysis of variance and two-tailed t-test were applied to the data. Protein was determined according to Lowry et al (1951).

Results

There was a significant ($p < 0.001$) decrease in the concentration of pyridoxal phosphate in various brain areas of pyridoxine-deficient rats as compared to controls. There was no significant difference in total protein or DNA content between the two groups. The concentration of serotonin in cerebral cortex of the deficient group was significantly ($p < 0.01$) reduced as compared to controls. However, there was no significant difference in the dopamine content of corpus striatum of the two groups (Table 1). The Bmax of serotonin binding increased significantly (193%, $p < 0.001$) with a significant decrease in binding affinity. Pyridoxine supplementation for one week reversed these alterations. No significant changes were seen in either parameter of [^3H]-spiroperidol binding in the striatum (Table 2).

Discussion

Changes in neurotransmitter receptor sensitivity have been reported in various diseases (Olsen et al 1980; Lozovsky et al 1981) as well as in partial starvation (Levin et al 1981). The increase in Bmax of serotonin binding seen in our study could be due to the chronically low intrasynaptic concentration of serotonin in the pyridoxine-deficient rat brain. The change in Kd indicates an alteration in the receptor protein as well. Multiple classes of both serotonin and dopamine receptors have been recognized (Peroutka et al 1981). The serotonin binding studied here would correspond to the S_1 subtype. It has been suggested that this site may be associated with 5HT sensitive adenylyl cyclase in the brain of young rats (Nelson et al 1980). The compensatory increase in binding to its receptors might still be inadequate for the normal function of the serotonergic pathway. We have also noted a significant decrease in the serotonin content with no change in the dopamine content of the hypothalamus of pyridoxine-deficient rats as compared to controls. The deficient rats had also significantly low levels of serum T_4 and T_3 as well as pituitary TSH. These results could be explained by the postulate of Smythe et al (1982) that serotonergic neurons are involved in thyrotropin secretion.

Conclusion

We conclude that there is a functional deficiency of serotonin in the pyridoxine deficient rat brain.

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Table 1
Effect of Pyridoxine Deficiency in 3 Week Old Rats on
Dopamine and Serotonin Levels

Status of animal	Dopamine in striatum nmoles/g wet weight*	Serotonin in cerebral cortex *
Pyridoxine-suppl. (Control)	11.5±2.2	1.15±0.17
Pyridoxine-def.	10.8±1.2	0.61±0.19**

* Means ± S.E.M. of 8 assays in each group

** p<0.01

Table 2
Effects of Pyridoxine Deficiency and Pyridoxine Supplementation
on [³H]-Serotonin Binding to Cerebral Cortex and [³H]-Spiroperidol
Binding to Striatal Membrane Preparations

Source of membranes	[³ H]-Serotonin binding		[³ H]-Spiroperidol binding	
	Bmax* fmoles/mg protein	Kd* nM	Bmax* fmoles/mg protein	Kd* nM
Pyridoxine-suppl. (Control 1)	838±45	14.1±0.17	126±16	0.51±0.05
Pyridoxine-def.	1836±64**	25.1±2.9**	123±14	0.57±0.01
Pyridoxine-suppl. 4th Wk. (Control 2)	888±56	15.4±1.9		

* Means ± S.E.M. of 8 assays in each group

** Statistically significant from both control groups.
p<0.001 (two-tailed t-test)

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