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Effect of Pyridoxine Deficiency in Young Rats on High-Affinity Serotonin and Dopamine Receptors

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The high-affinity bindings of [^{3}H]-5-hydroxytryptamine to serotonin S-1 receptors, [^{3}H]-ketanserin to serotonin S-2 receptors in the cerebral cortex, [^{3}H]-fluphenazine to dopamine D-1 receptors, and [^{3}H]-spiroperidol to dopamine D-2 receptors in the corpus striatum were studied in pyridoxine-deficient rats and compared to pyridoxine-supplemented controls. There was a significant increase in the maximal binding (B_{max}) of serotonin S-1 and S-2 receptors with a significant decrease in their binding affinities (K_d). However, there were no significant changes either in the maximal binding or binding affinity of striatal dopamine D-1 and D-2 receptors. Receptor sensitivity seems to correlate negatively with the corresponding neurotransmitter concentrations in the pyridoxine-deficient rats.

Key words: serotonin, dopamine, receptors, pyridoxine deficiency

INTRODUCTION

Changes in neurotransmitter receptor sensitivity, measured by kinetic parameters, maximal binding (B_{max}), and binding affinity (K_d), have been reported in various diseases [Conti-Tronconi et al, 1980; Reynolds et al, 1984], as well as during dietary restriction or perinatal undernutrition [Keller et al, 1982]. Experimental conditions that increase intrasynaptic neurotransmitter concentration decrease postsynaptic receptor sensitivity, and conversely conditions that decrease neurotransmitter concentration lead to enhanced postsynaptic sensitivity [Bloom et al, 1981]. We have reported earlier that the contents of γ-aminobutyric acid (GABA) and serotonin (5-HT) in the brain of pyridoxine-deficient rats are significantly decreased, whereas the brain contents of the catecholamines are unaffected [Stephens et al, 1971; Dakshinamurti et al, 1976]. Recently we also reported that there is an increase of high-affinity GABA receptor binding in the cerebellum of pyridoxine-deficient rat [Paulose and Dakshinamurti, 1984]. In the present investigation the effects of pyridoxine deficiency on

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dopamine, its high-affinity D-1 and D-2 receptors in the corpus striatum and serotonin, and its high affinity S-1 and S-2 receptors in the cerebral cortex of rat brain have been studied.

MATERIALS

Sprague-Dawley rats purchased from the Canadian Breeding Farm (St. Constant, Quebec) were used in these studies. [^{3}H]-5-Hydroxytryptamine (30.3 Ci/mmol), [^{3}H]-ketanserin (66.9 Ci/mmol), [^{3}H]-spiroperidol (34.0 Ci/mmol), and [^{3}H]-fluphenazine (35.5 Ci/mmol) were purchased from New England Nuclear (Boston, MA). All other chemicals used were purchased from Sigma (St. Louis, MO). Spiroperidol and Ketanserin were a gift from Janssen pharmaceuticals (Beerse, Belgium). Fluphenazine was a gift from Schering Canada (Quebec).

METHODS

The objective of this study was to compare pyridoxine-deficient pups with pups that had gone through an equivalent period of generalized malnutrition while getting adequate pyridoxine (control) and with pups on ad libitum normal diet (normal). Thus, any difference between the deficient and control pups could be ascribed directly to their pyridoxine status.

Sperm-positive Sprague-Dawley rats were housed individually and fed a pyridoxine-supplemented diet containing 100 mg pyridoxine added per 1 kg of deficient diet for the period of gestation. At the time of delivery the dams were divided into three groups. The number of pups left with the dam was manipulated soon after birth so that each dam in the deficient (fed the pyridoxine-deficient diet) and normal (fed the pyridoxine-supplemented diet) groups had eight pups. In the control group (fed the pyridoxine-supplemented diet) each dam was left with 16 pups. The percentage composition of the pyridoxine-deficient diet [Dakshinamurti and Stephens, 1969] was as follows: vitamin-free casein, 30.0; dextrose, 59.85; corn oil, 5.0; salt mix, No. 446, 4.0; vitamin mix without pyridoxine, 1.0; and choline chloride, 0.15. All dams were fed the respective diets ad libitum. However, because of the large number per litter, the pups in the control group were getting less milk and thus were subjected continually to a generalized malnutrition so that their body weights were close to those of deficient pups. Pups from all the groups were killed when they were 21 days old. The brain regions were dissected according to Glowinski and Iversen [1966]. Tissues were frozen immediately on dry-ice and stored at $-70°C$. Cerebral cortex was used for measuring pyridoxal phosphate, serotonin, and the kinetic parameters of serotonin S-1 and S-2 receptors. Corpus striatum was used for measuring pyridoxal phosphate, dopamine, and kinetic parameters of dopamine D-1 and D-2 receptors. Pyridoxal phosphate was determined using tyrosine apodecarboxylase, as described previously [Dakshinamurti and Stephens, 1969]. The radioenzymatic method of Hammel et al [1978] was used for the assay of serotonin. A similar procedure of Sole and Hussain [1977] was used for determining dopamine content.

The crude synaptic membranes from cerebral cortex and corpus striatum for binding studies were prepared by the following procedure. The tissue was homogenized in 50 mM Tris-HCl buffer with polytron homogenizer (setting at 6) for 30 sec. The homogenate was centrifuged at 48,000g for 15 min. The pellet was resuspended

in the same buffer and the procedure was repeated twice. The final pellet was resuspended in the assay buffer and was used for binding studies.

[^{3}H]-5-Hydroxytryptamine binding was assayed in the cerebral cortex membrane preparations by the method of Uzbekov et al [1979]. [^{3}H]-Ketanserin binding was assayed according to Leysen et al [1982] in the cerebral cortex membrane preparations.

The binding of [^{3}H]-spiroperidol and [^{3}H]-fluphenazine were determined in the striatal membrane preparations according to Creese et al [1978]. Specific binding data were analyzed according to Scatchard [1949], from which maximal binding (B_{max}) and the dissociation constant (K_d) were derived by linear regression analysis. The data were analyzed statistically by analysis of variance and two-tailed t test. Protein was measured according to Lowry et al [1951]. DNA was estimated according to Schneider [1957].

RESULTS

Earlier findings [Stephens et al, 1971] and the present observations clearly show a significant decrease in body and brain weights of the pyridoxine-deficient (D) rats compared to the pyridoxine-supplemented controls (C) and normals (N). Body weight: normal, 58 ± 4.3 g; control, 45 ± 4.8 g; pyridoxine-deficient, 31 ± 2.3 g, ($P < 0.001$, D compared with N and C). Brain weight: normal, 1.59 ± 0.04 g; control, 1.54 ± 0.03 g; pyridoxine-deficient, 1.28 ± 0.03 g, ($P < 0.001$, D compared with N and C). There were no significant differences in the contents of total protein or DNA in the corpus striatum and cerebral cortex of pyridoxine-deficient, control, and normal groups. There was no significant difference between normal and control groups in the contents of pyridoxal phosphate, serotonin, and dopamine in various brain regions. The contents of pyridoxal phosphate in cerebral cortex and corpus striatum of deficient rats were 43% and 29%, respectively, of control values. The content of serotonin in cerebral cortex of deficient rats was 53% of control. There was no significant difference between the two groups in the dopamine content in corpus striatum (Table I). In receptor binding studies the deficient group was compared to the control, as there was no difference between the control and normal groups in their neurotransmitter contents.

The Scatchard analysis of [^{3}H]-5-hydroxytryptamine binding to membrane preparations from cerebral cortex of deficient and control rats given in Figure 1 indicates an increase in serotonin S-1 receptor concentration ($P < 0.001$) with a significantly ($P <$

TABLE I. Pyridoxal Phosphate, Serotonin, and Dopamine Contents (nmoles/gm wet wt) of 3-Week-Old Rats*

Experimental group	Pyridoxal phosphate in cerebral cortex	Serotonin in cerebral cortex	Pyridoxal phosphate in corpus striatum	Dopamine in corpus striatum
Normal	3.61 ± 0.16	1.12 ± 0.13	3.28 ± 0.25	11.46 ± 1.81
Control	3.74 ± 0.16	1.15 ± 0.17	3.48 ± 0.36	11.51 ± 2.16
Pyridoxine-deficient	1.72[a] ± 0.09	0.61[b] ± 0.19	1.00[a] ± 0.10	10.75 ± 1.22

*Data are mean ± SEM, determined from eight separate experiments each assayed in triplicate.
[a]$P < 0.001$.
[b]$P < 0.01$ with respect to control and normal.

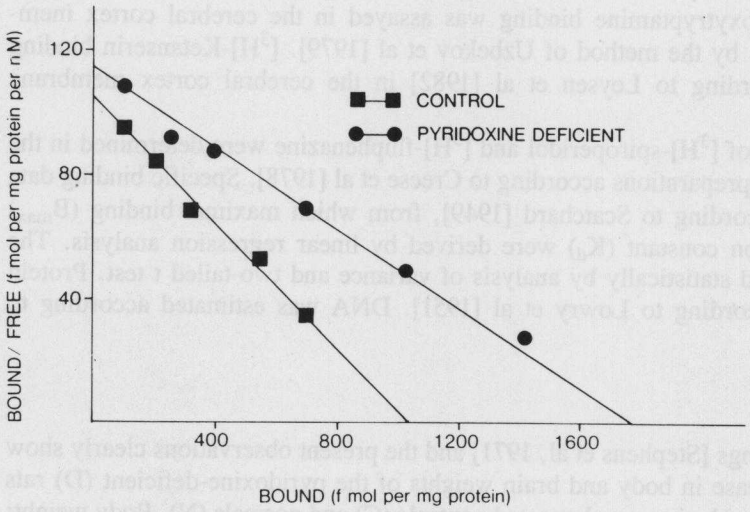

Fig. 1. Scatchard analysis of [^{3}H]-5-hydroxytryptamine binding in crude synaptic membrane preparations from the cerebral cortex of 21-day-old rats. Crude synaptic membrane preparation was suspended in 50 mM Tris-HCl buffer (pH 8.5) containing 1.0 μM pargyline, and 0.3–0.4 mg protein was used in each assay. The incubation mixture containing 1–30 nM [^{3}H]-5HT with and without excess of unlabeled 5-HT (10 μM) was incubated for 15 min at 37°C. The contents of the incubation tubes were rapidly filtered under partial vacuum through GF/B filters and washed 3 × with 5 ml ice-cold 50 mM Tris-HCl buffer (pH 8.5). [^{3}H]-HT bound to the membranes in the filter was determined by liquid scintillation spectrometry. Specific binding was determined by subtracting nonspecific binding from the total binding.

TABLE II. [^{3}H]-5-Hydroxytryptamine and [^{3}H]-Ketanserin Binding in the Cerebral Cortex of 3-Week-Old Rats*

	[^{3}H]-5-Hydroxytryptamine binding		[^{3}H]-Ketanserin binding	
Experimental group	Bmax (fmoles/mg protein)	Kd (nM)	Bmax (fmoles/mg protein)	Kd (nM)
Control	934 ± 44	10.02 ± 0.78	217 ± 18	0.69 ± 0.07
Pyridoxine-deficient	1799[a] ± 129	20.12[b] ± 2.06	306[c] ± 21	1.09[c] ± 0.11

*Data are mean ± SEM, determined from eight separate experiments each assayed in triplicate.
[a]$P < 0.001$ with respect to control.
[b]$P < 0.01$ with respect to control.
[c]$P < 0.025$ with respect to control.

0.01) decreased binding affinity (Table II). The similar analysis of [^{3}H]-ketanserin binding to cerebral cortex membrane preparations given in Figure 2 and Table II shows an increase in B_{max} ($P < 0.025$) with a lowered ($P < 0.025$) binding affinity. Membrane preparations from corpus striatum of deficient and control rat brains have been used to study the bindings, respectively, of [^{3}H]-fluphenazine and [^{3}H]-spiroperidol. There was no significant difference between pyridoxine-deficient and control groups either in the receptor concentration or in the receptor binding affinities (Table III).

DISCUSSION

We have reported a significant decrease in total brain level of serotonin with no change in the level of catecholamines in the pyridoxine-deficient rat [Dakshinamurti

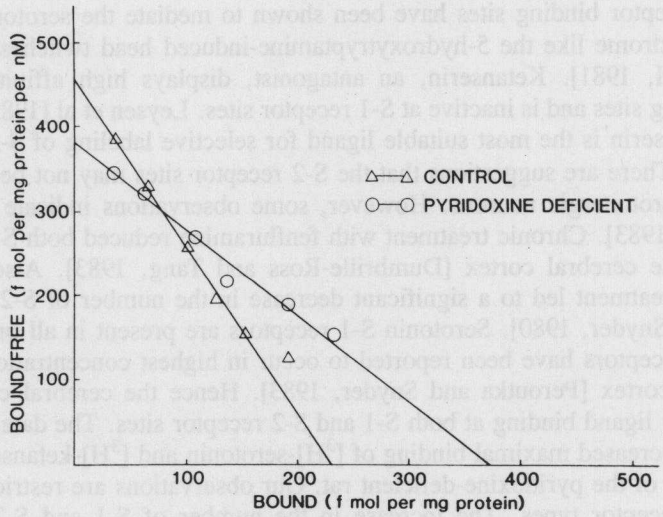

Fig. 2. Scatchard analysis of [^{3}H]-ketanserin binding in crude synaptic membrane preparations from the cerebral cortex of 21-day-old rats. Crude synaptic membrane preparation was suspended in 50 mM Tris-HCl buffer (pH 7.6), and 0.3–0.4 mg protein was used in each assay. The incubation mixture containing 0.1–2 nM [^{3}H]-ketanserin with and without excess of unlabeled ketanserin (1 μM) was incubated for 15 min at 37°C. The contents of the incubation tubes were rapidly filtered under partial vacuum through GF/B filters and washed 3 × with 5 ml ice-cold 50 mM Tris-HCl buffer (pH 7.6). [^{3}H]-Ketanserin bound to the membranes in the filter was determined by liquid scintillation spectrometry. Specific binding was determined by subtracting nonspecific binding from the total binding.

TABLE III. [^{3}H]-Spiroperidol and [^{3}H]-Fluphenazine Binding in the Corpus Striatum of 3-Week-Old Rats*

Experimental group	[^{3}H]-Spiroperidol binding		[^{3}H]-Fluphenazine binding	
	B_{max} (fmoles/mg protein)	K_d (nM)	B_{max} (fmoles/mg protein)	K_d (nM)
Control	126 ± 16	0.51 ± 0.05	277 ± 14	0.98 ± 0.07
Pyridoxine-deficient	123 ± 14	0.57 ± 0.06	287 ± 15	1.05 ± 0.07

*Data are mean ± SEM, determined from eight separate experiments each assayed in triplicate.

et al, 1976]. Various studies including loading with 5-hydroxytryptophan have indicated that in pyridoxine deficiency the decarboxylation step is affected. The decreased motility and hypothermia seen in the pyridoxine-deficient rat could be the result of a functional deficit of the serotonergic system. The duration of deep slow-wave sleep 2 and REM sleep were shortened and in some instances completely abolished in the pyridoxine-deficient rat [Dakshinamurti, 1982]. Sleep studies indicate a parallel between the effects of pyridoxine deficiency and serotonin deficiency [Kiianmaa and Fuxe, 1977]. Results presented here indicate that although the pyridoxal phosphate level is decreased in both the cerebral cortex and the corpus striatum this decrease affects only the formation of serotonin. The effect of a chronic deficiency of serotonin on the neurotransmitter receptors has now been examined. Two distinct types of serotonin receptor binding sites have been recognized in in vitro binding studies [Peroutka and Snyder, 1983]. [^{3}H]-Serotonin appears to be the only selective ligand labeling S-1 receptor binding sites [Fillion et al, 1978]. A relationship between these

sites and serotonin-sensitive adenylate cyclase has been proposed [Peroutka et al, 1981]. S-2 receptor binding sites have been shown to mediate the serotonin-related behavioral syndrome like the 5-hydroxytryptamine-induced head twitches in the rat [Peroutka et al, 1981]. Ketanserin, an antagonist, displays high affinity for S-2 receptor binding sites and is inactive at S-1 receptor sites. Leysen et al [1982] indicate that [^{3}H]-ketanserin is the most suitable ligand for selective labeling of S-2 receptor binding sites. There are suggestions that the S-2 receptor sites may not be under the influence of serotonergic neurons. However, some observations indicate otherwise [Schotte et al, 1983]. Chronic treatment with fenfluramine reduced both S-1 and S-2 receptors in the cerebral cortex [Dumbrille-Ross and Tang, 1983]. Also, chronic amitriptyline treatment led to a significant decrease in the number of S-2 receptors [Peroutka and Snyder, 1980]. Serotonin S-1 receptors are present in all brain areas, whereas S-2 receptors have been reported to occur in highest concentrations, in the rat prefrontal cortex [Peroutka and Snyder, 1983]. Hence the cerebral cortex was chosen to study ligand binding at both S-1 and S-2 receptor sites. The data presented here indicate increased maximal binding of [^{3}H]-serotonin and [^{3}H]-ketanserin in the cerebral cortex of the pyridoxine-deficient rat. Our observations are restricted to the high-affinity receptor types. The increase in the number of S-1 and S-2 receptor binding sites might be the result of chronic low intrasynaptic concentration of serotonin in the pyridoxine-deficient rat. The significantly lowered binding affinity of the ligands studied would indicate a possible alteration in the receptor proteins as well. It is possible that other factors not related to neurotransmitter deficiency might alter the capacity to produce receptors. Although there is a compensatory increase in the number of S-1 and S-2 binding sites in the cerebral cortex of the pyridoxine-deficient rat, serotonergic neurotransmission and its physiologic function seem to be impaired in these animals in view of the low synaptic concentration of the neurotransmitter. The hypothyroidism of pyridoxine-deficient young rats has been ascribed to the decrease in serotonin, with no change in the dopamine content of the hypothalamus of these animals [Dakshinamurti et al, 1985].

The presence of at least two or possibly three distinct classes of dopaminergic binding sites in corpus striatum, the most commonly studied tissue for dopamine receptor binding, has been recognized [Creese and Leff, 1982]. Phenothiazine antagonists like fluphenazine [Clement-Cormier et al, 1974] antagonize dopamine-stimulated cAMP formation and label both D-1 and D-2 receptors in the striatum. [^{3}H]-Spiroperidol, a butyrophenone neuroleptic, labels the D-2 receptor sites. Spiroperidol is a very potent antagonist of D-2 receptor but exhibits only weak affinity for D-1 receptors. We have examined the high-affinity binding of [^{3}H]-spiroperidol and [^{3}H]-fluphenazine to striatal membrane dopamine receptors. There was no significant difference between pyridoxine-deficient and control animals in regard to any of the binding parameters for either ligand. This correlated well with the lack of any significant difference between these two groups of rats in regard to the content of dopamine in the striatum. The results presented here do indicate that receptor sensitivity is modulated by the synaptic concentration of the corresponding neurotransmitter.

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