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Neuroendocrinology of Pyridoxine Deficiency

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DAKSHINAMURTI, K., C. S. PAULOSE, M. VISWANATHAN AND Y. L. SIOW. *Neuroendocrinology of pyridoxine deficiency.* NEUROSCI BIOBEHAV REV 12(3/4) 189–193, 1988.— Dihydroxyphenylalanine decarboxylase and 5-hydroxytryptophan decarboxylase respectively have high and low affinities for pyridoxal phosphate. In the pyridoxine-deficient animal, hypothalamic serotonin content is significantly reduced without any change in catecholamine levels. Hypothalamic neurotransmitters affect the hypothalamo-pituitary-end organ axes. Specifically, the decrease in hypothalamic serotonin in the pyridoxine-deficient rat results in tertiary hypothyroidism. In addition, pineal function is affected in deficient animals due to decreased synthesis of melatonin.

Decarboxylase Serotonin Melatonin Hypothyroidism

PYRIDOXINE plays a central role in the metabolism of the nervous system. Pyridoxal 5′-phosphate (PLP) is the major coenzyme form of pyridoxine and participates as such in over 50 enzymatic reactions, mostly in the metabolism of various amino acids. Pyridoxamine 5′-phosphate can catalyze amino transferase reactions by the cyclic regeneration of the two active phosphate forms of pyridoxine. The putative neurotransmitters, dopamine, norepinephrine, serotonin, gamma aminobutyric acid, and taurine, as well as the sphingolipids and polyamines, are synthesized by pyridoxine-dependent enzymes. Of these, three enzymes, viz, glutamic acid decarboxylase, 5-hydroxytryptophan (5-HTP) decarboxylase, and ornithine decarboxylase are crucial and can explain most of the neurological defects of pyridoxine deficiency in animals.

The synthesis of dopamine and serotonin involves a pyridoxal phosphate-dependent decarboxylation step. This is generally considered to be catalyzed by the enzyme aromatic amino acid decarboxylase (EC 4.1.1.28) which lacks substrate specificity. This has been considered to be a single protein entity based primarily on the evidence of Christenson *et al.* (5) who raised a goat antiserum against purified pig kidney enzyme and found that this antiserum precipitated material active toward both dihydroxyphenylalanine (DOPA) and 5-HTP to equal extents. However, the recent demonstration by Ando-Yamamoto *et al.* (1) of immunochemical cross-reactivity of DOPA decarboxylase and histidine decarboxylase using antibodies against these two enzymes suggests the presence of similar antigenic recognition sites inside the native molecules of the decarboxylases that are exposed when the enzymes are denatured. There are many differences in the optimal conditions, kinetics, affinity for PLP, activation and inhibition by specific chemicals and regional differences in the distribution of DOPA and 5-HTP decarboxylases (22–24). Sourkes (26) has stated "one could just as well postulate the existence of two homologous enzymes possessing equivalent structure and conformation at the immunocompetent regions of the peptide chain." During the course of our purification of DOPA decarboxylase from bovine striatum we noticed preferential enrichment of DOPA decarboxylase activity as against 5-HTP decarboxylase activity. Earlier, we have reported on nonparallel changes in brain monoamines in pyridoxine deficiency (7). Only serotonin (5-HT) was decreased whereas dopamine and norepinephrine were not affected in pyridoxine deficiency. This is in keeping with the higher affinity of DOPA decarboxylase as compared to 5-HTP decarboxylase for pyridoxal phosphate (23). The specificity of the decrease in serotonin and its relationship to the pyridoxine status of the animal was established. We excluded possibilities such as decreased precursor availability or increased catabolism. Also loading experiments using the immediate precursor 5-hydroxytryptophan have shown that the decarboxylation step is the site of difference between the pyridoxine-deficient and pyridoxine-supplemented rats.

We have examined the possibility that the decrease in serotonin in various brain areas of the pyridoxine-deficient rat has physiological consequences. Applying the pharmacological principle that experimental conditions which increase intrasynaptic neurotransmitter concentration decrease postsynaptic receptor sensitivity and conversely so, we investigated the kinetic parameters of receptor binding of the ligands [^{3}H] 5-HT and [^{3}H] ketanserin to the respective receptors in synaptosomal membrane preparations of cerebral cortex from pyridoxine-deficient rats (20). Significant increases in B_{max} of serotonin S1 and S2 receptors were seen. However the B_{max} and binding affinities of the ligands

TABLE 1

PYRIDOXAL PHOSPHATE, SEROTONIN, DOPAMINE AND NOREPINEPHRINE CONTENTS IN CONTROL AND PYRIDOXINE-DEFICIENT RAT HYPOTHALAMUS

Animal Status	Pyridoxal Phosphate (nmol/g)	Serotonin (nmol/g)	Dopamine (nmol/g)	Norepinephrine (nmol/g)
Pyridoxine-supplemented (control)	2.71 ± 0.19	1.70 ± 0.20	1.18 ± 0.07	2.17 ± 0.09
Pyridoxine-deficient	1.17 ± 0.07†	1.00 ± 0.27*	1.25 ± 0.10	2.01 ± 0.10

*$p<0.01$, †$p<0.001$ compared with controls (Student's unpaired t-test). [Used with permission from publisher (8).]

Values are means ± S.E.M. of eight separate determinations in each group.

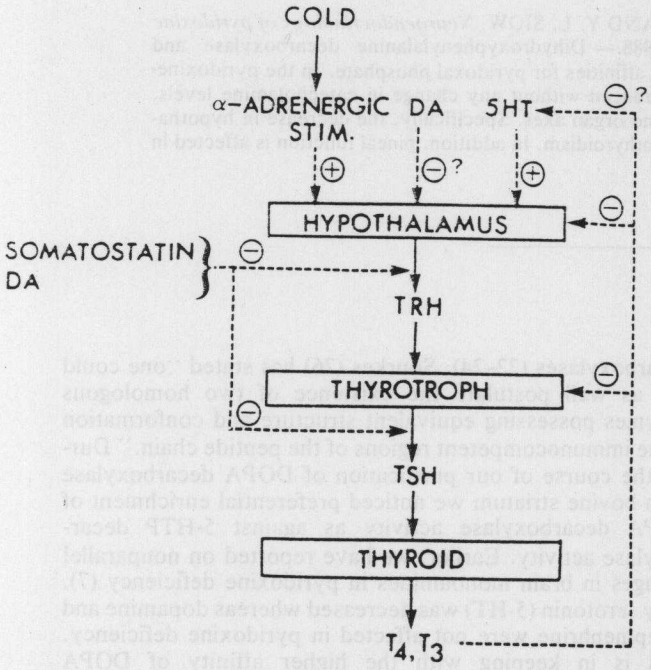

FIG. 1. Hypothalamus-pituitary-thyroid relationship.

to the respective dopamine D-1 and D-2 receptors were not affected in deficient synaptosomal membrane preparations. The supersensitivity of $GABA_A$ and $GABA_B$ receptors in synaptosomal membrane preparations from pyridoxine-deficient rat cerebellum correlated negatively with the concentration of GABA in cerebellum of these animals (19). Decreased brain serotonin in the pyridoxine-deficient rat is implicated in various physiological processes like the decrease in deep body temperature and motility of these animals. Also, the duration of deep slow-wave sleep 2 (SWS 2) is shortened and in some instances this stage of sleep is completely abolished. REM sleep is also affected similarly and the deficient animals are in shallow slow-wave sleep (SWS 1) (6). The effects of pyridoxine deficiency on sleep parallel the effects of experimental serotonergic deficit (11).

The hypothalamus is one of the brain areas of the pyridoxine-deficient rat with a considerable decrease in both pyridoxal phosphate and serotonin (Table 1). The secretion by the anterior pituitary of ACTH, growth hormone, prolactin, thyroid stimulating hormone (TSH), and the gonadotropins is governed by releasing factors and in some instances by release of inhibiting factors from the hypothalamus. This concept of the regulatory role of the hypothalamus and brain neurotransmitters has generally been accepted. Regulation of the release of these factors from the hypothalamus involves complex neural circuitry in which the serotonergic neurons represent one link in the control mechanism. Both dopamine and serotonin are present in high concentration in the hypothalamus and are essentially antagonistic in their effect on pituitary hormone regulation (12).

We have examined the hypothalamus-pituitary-thyroid relationship (Fig. 1). The secretion of TSH is directly controlled by two factors—a negative feedback signal indicating serum thyroid status and a stimulatory factor, thyrotropin releasing hormone (TRH) released from the hypothalamus. The postulate that serotonergic neurons stimulate TSH secretion in rats is supported by the observation that injection of serotonin into the third ventricle caused rapid increase in serum TSH. This effect was completely reversed by pretreatment of rats with cyproheptidine, a serotonin receptor antagonist. Krulich *et al.* (12), on the other hand, found decrease of serum TSH in rats after intraventricular injection of serotonin. Other reports (4) indicate that serotonin stimulates TRH release from the superfused hypothalamus. Smythe *et al.* (25) have demonstrated a direct relationship between hypothalamic serotonin turnover and TSH release. The dopaminergic neurons exert an inhibitory effect on the secretion of TSH. This effect is at the level of the pituitary itself as bromocriptine blunts the stimulatory effect of TRH in euthyroid subjects (3). The inhibitory effect of dopamine is abolished by dopamine receptor antagonists like domperidone (10). It is well recognized that the cold-induced TSH secretion is mediated by norepinephrine (13). Pharmacological studies using inhibitors of norepinephrine synthesis or alpha-adrenergic blockers like phentolamine indicate a stimulatory role for norepinephrine in the control of TRH-mediated TSH secretion (17). The contradictory stimulatory effect of small doses of alpha-adrenergic antagonists might be explained on the basis of alpha-adrenergic receptor subtypes. $Alpha_1$ is inhibitory and $alpha_2$ is stimulatory (15). On balance it appears that serotonergic neurons have a stimulatory effect on hypothalamic control of

TABLE 2

SERUM T_4, T_3, TSH AND PITUITARY TSH IN NORMAL, CONTROL AND PYRIDOXINE-DEFICIENT 3-WEEK-OLD RATS

	Normal (Group 1)	Control (Group 2)	Pyridoxine-Deficient (Group 3)
T_4 (nmol/l)	87.52 ± 3.76 (19)	82.98 ± 1.88 (40)	58.39 ± 2.66† (41)
T_3 (nmol/l)	1.54 ± 0.07 (20)	1.40 ± 0.06 (42)	0.98 ± 0.03† (41)
Serum TSH (μg/l)	2.63 ± 0.15 (16)	2.75 ± 0.16 (22)	2.45 ± 0.18 (15)
Pituitary TSH (μg/mg protein)	6.00 ± 0.38 (17)	8.21 ± 0.39* (41)	4.68 ± 0.32† (38)
Pituitary TSH (μg/pituitary)	2.12 ± 0.15 (17)	1.80 ± 0.12 (41)	1.05 ± 0.05† (38)

*$p<0.05$ compared with Group 1; †$p<0.01$ compared with Group 1 and Group 2 respectively (Duncan's multiple range test). [Used with permission from publisher (8).] Values are means ± S.E.M.; number of determinations are given in parenthesis.

TABLE 3

EFFECTS OF TRH AND T_4 ON PITUITARY TSH, AND SERUM TSH, T_4 AND T_3 IN PYRIDOXINE-SUPPLEMENTED AND PYRIDOXINE-DEFICIENT 3-WEEK-OLD RATS

Treatment	Pituitary TSH (μg/mg protein)	Pituitary TSH (μg/pituitary)	Serum TSH (μg/l)	Serum T_4 (nmol/l)	Serum T_3 (nmol/l)
Saline					
Group 1	6.83 ± 0.14 (11)	1.09 ± 0.04 (11)	1.74 ± 0.13 (9)	81.21 ± 1.92 (14)	1.51 ± 0.08 (14)
Group 2	4.12 ± 0.15† (8)	0.81 ± 0.05† (8)	1.92 ± 0.23 (13)	52.00 ± 2.95* (16)	0.96 ± 0.05* (16)
TRH					
Group 1	4.55 ± 0.14§ (12)	0.74 ± 0.03§ (12)	5.54 ± 0.76§ (11)	104.29 ± 7.70‡ (7)	1.76 ± 0.08 (7)
Group 2	5.88 ± 0.15†§ (10)	1.04 ± 0.05† (10)	5.82 ± 0.57§ (7)	86.29 ± 4.66*‡ (7)	2.37 ± 0.20*§ (7)
T_4					
Group 1	1.28 ± 0.08§ (7)	0.20 ± 0.02§ (7)	1.13 ± 0.05‡ (7)	901 ± 29§ (7)	24.86 ± 1.22§ (7)
Group 2	3.76 ± 0.34† (5)	0.62 ± 0.05†‡ (5)	1.23 ± 0.13‡ (6)	1330 ± 104†§ (5)	21.60 ± 1.03†§ (5)

*$p<0.05$, †$p<0.01$ compared with Group 1. ‡$p<0.05$, §$p<0.01$ compared with saline-treated groups (Duncan's multiple range test). [Used with permission from publisher (9).] Values are means ± S.E.M.; number of determinations are given in parenthesis; Group 1: pyridoxine-supplemented; Group 2: pyridoxine-deficient.

pituitary secretion of TSH in circumstances where central control is natural like in timing of the circadian rhythm and possibly in the pulsatile secretion of TSH (16).

In view of this we compared the thyroid status of pyridoxine-deficient and pyridoxine-supplemented rats (8). These experiments included 3-week-old deficient, normal and a control group of rats on a pyridoxine-supplemented diet whose food intake was adjusted so that they pair-weighed with the deficient rats. Serum concentrations of thyroxine (T_4) and tri-iodothyronine (T_3) of the deficient group were significantly lower in comparison with normal and control rats. There was no significant change in the concentration of the serum TSH in the deficient group (Table 2). However, deficient rats had significantly lower content of pituitary TSH. These experiments were repeated later using moderately pyridoxine-deficient adult rats with appropriate controls (21). Pyridoxine treatment restored to normal the hypothalamic levels of pyridoxal phosphate and serotonin.

In determining the locus of the biochemical lesion leading to the hypothyroid state in the pyridoxine deficiency, various possibilities—primary with a defective thyroid gland, secondary with a defective pituitary thyrotroph or tertiary

TABLE 4

EFFECT OF PYRIDOXINE DEFICIENCY IN ADULT MALE RATS ON THE CIRCADIAN MELATONIN (MT) CONCENTRATION IN SERUM

	Serum MT (pg/ml)		Pineal MT (pg/gland)	
	Day	Night	Day	Night
Pyridoxine-supplemented (control)	<5	29.3 ± 0.5	42.3 ± 0.27	1923 ± 452
Pyridoxine-deficient	7.7 ± 2	13.4 ± 1.8*	60.3 ± 9.2	1150 ± 139*

*$p<0.05$ compared with pyridoxine-supplemented (control).
Values are means ± S.E.M. of 8 separate determinations in each group.

with a defect in the hypothalamus—have to be considered. If the defect were only at the level of the thyroid gland low serum T_4 and T_3 values would be coupled with a compensatory rise in serum TSH which was not seen. With a defective pituitary the low levels of serum T_3 and T_4 values would be coupled with a sharp decrease in serum TSH as well as unresponsiveness to TRH. This, again, was not seen in the deficient rats. Hypothalamic hypothyroidism is due to deficient TRH secretion. In examining this possibility Dakshinamurti *et al.* (9) injected TRH (15 μg/100 g body weight, IP every day for 7 days) to pyridoxine-deficient and control (pyridoxine-supplemented) rats. The effect of the vehicle (saline) alone was also assessed. TRH treatment significantly increased serum TSH as well as serum T_4 and T_3 in both pyridoxine-deficient and control rats (Table 3).

We have also studied the kinetics of ligand binding, using [^{3}H]-methyl histidine analog of TRH, in membrane preparations from pyridoxine-deficient and control pituitaries. Scatchard analysis of the binding data indicates an increase in the number of receptors with no change in receptor affinity in the deficient pituitary membrane preparations (9). This would be a reflection of the chronic deficiency of TRH exposed to its receptor on the pituitary thyrotroph of the pyridoxine-deficient rat. These results can be interpreted as consistent with hypothalamic type of hypothyroidism in the pyridoxine-deficient rat caused by the specific decrease in hypothalamic serotonin.

Another neuroendocrine system to be considered is the pineal, an organ present in the brain but anatomically not a part of the central nervous system. Increasing attention is now being given to functions other than the antigonadotropic ones of melatonin, one of the major secretory products of pineal. Melatonin is formed from serotonin in two steps. Serotonin is first converted to N-acetyl serotonin which is then methylated in the 5-hydroxy position to yield melatonin. There is a circadian rhythm in the formation and secretion of melatonin. We have examined the effect of pyridoxine deficiency on indoleamine metabolism in the pineal gland. Male Sprague-Dawley rats (150–160 g) were fed either a pyridoxine-supplemented or pyridoxine-deficient diet for 8 weeks. The animals were maintained on a 14:10 L:D cycle. The pineal as well as serum melatonin levels were highest at night, measured between midnight and 2 a.m., and lowest correspondingly during the day, measured between noon and 2 p.m. (Table 4). Pyridoxine deficiency did not alter the pattern of circadian variation in serotonin and melatonin. There were significant decreases in pineal and serum contents of melatonin in the pyridoxine-deficient rat. The levels of serotonin and its metabolite 5-hydroxyindoleacetic acid—taken together as a representation of the serotonergic pathway—were decreased in the pineal gland of the pyridoxine-deficient rat. They were partly restored within 24 hours following pyridoxine administration to the deficient young rat. Similar data were obtained with pyridoxine-deficient and control adult rats as well. The synthesis and secretion of melatonin is under sympathetic control. However, there was no difference in the norepinephrine contents of the pineals of pyridoxine-deficient and pyridoxine-supplemented control rats, and pyridoxine treatment, as expected, did not alter these levels. We contend that changes in melatonin seen in the pyridoxine-deficient rat are related to the decrease in serotonin, the precursor of melatonin. It is to be noted that changes are seen even in the moderately pyridoxine-deficient adult rat.

The possibility of a connection between pineal function and neuropsychiatric illness like depression has been suspected for some time (27). The metabolism of melatonin is affected by antidepressants (18). Neuropsychiatric disorders such as changes in sleep pattern and mood seen in a small group of women using oral contraceptive steroids are reported to be corrected by high doses of pyridoxine (2). The implications of mild pyridoxine deficiency on pineal function take added significance in this context.

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