Neurobiology of Pyridoxine[a]

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The diversity of biochemical reactions involving the coenzymatic forms of pyridoxine (vitamin B_6) is well recognized. There are over 100 pyridoxal phosphate (PLP) dependent enzymes. Most are involved in catabolic reactions of various amino acids. The crucial role played by pyridoxine in the nervous system is evident from the fact that the putative neurotransmitters, dopamine (DA), norepinephrine (NE), serotonin (5-HT), γ-aminobutyric acid (GABA), and taurine as well as the sphingolipids and polyamines are synthesized by PLP-dependent enzymes. Considerable variation exists in the affinities of different apoenzymes for PLP. This explains the observed differential susceptibility of various PLP enzymes to decrease during the progression of pyridoxine deficiency.[1] Of the pyridoxine enzymes, three, namely, glutamic acid decarboxylase, 5-hydroxytryptophan decarboxylase, and ornithine decarboxylase, are crucial and can explain most of the neurological defects of pyridoxine deficiency in animals.[2,3] This presentation will focus on the role of pyridoxine in the control of hypothalamo-pituitary-end organ systems, melatonin synthesis, and convulsive seizure activity.

The enzyme L-aromatic amino acid decarboxylase (EC 4.1.1.28), which lacks substrate specificity, has been considered to be involved in the formation of the catecholamine as well as serotonin.[3] This has been suggested to be a single protein entity, based on immunological evidence of Christenson *et al.*[4] However, the recent demonstration by Ando-Yamamoto *et al.*[4] of immunological cross-reactivity of dihydroxyphenylalanine (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 for enzyme activity, including kinetics, affinity for PLP, activation and inhibition by specific chemicals, and regional differences in the distribution of DOPA and 5-hydroxytryptophan (5-HTP) decarboxylases.[6-8] During the course of purification of DOPA decarboxylase from bovine striatum, there was a preferential enrichment of

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DOPA decarboxylase activity as against 5-HTP decarboxylase activity.[9] Earlier, we have reported on nonparallel changes in brain monamines in the pyridoxine-deficient rat.[10] Brain contents of dopamine and norepinephrine were not affected in deficiency, whereas serotonin was decreased. 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. Loading experiments using the immediate precursor, 5-HTP, have shown that the decarboxylation step is the site of difference between the pyridoxine-deficient and pyridoxine-supplemented rats.

The decrease in serotonin in various brain areas of the pyridoxine-deficient rat has physiological consequences. That there was a decrease in the synaptic release of serotonin in the deficient rat brain regions was indicated by the increase in the postsynaptic receptor density. Significant increases were seen in the B_{max} of the ligands for serotonin S_1 and S_2 receptors in synaptosomal membrane preparations of cerebral cortex from pyridoxine-deficient rats.[11] The B_{max} and binding affinities of the ligands to the respective dopamine D-1 and D-2 receptors were not affected in synaptosomal membrane preparations from pyridoxine-deficient rat striatum. This is in keeping with the data on dopamine levels. 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 the cerebellum of the animals.[12] Decreased brain serotonin in the pyridoxine-deficient rat is implicated in physiological changes such as decreased deep-body temperature and altered sleep pattern with shortening of deep slow-wave sleep[2] and REM sleep.[2] The effects of pyridoxine deficiency on sleep paralleled the effects of experimental serotonergic deficit.[13]

HYPOTHALAMO-PITUITARY-END ORGAN RELATIONSHIP

The hypothalamus is one of the brain areas of the pyridoxine-deficient rat with significant decreases in pyridoxal phosphate and serotonin as compared with pyridoxine-supplemented controls. The decrease in pyridoxal phosphate in the deficient hypothalamus did not result in any decrease in the contents of dopamine and norepinephrine (TABLE 1). However, there was a significant decrease in the serotonin content. The secretion by the anterior pituitary of ACTH, growth hormone, prolactin, thyroid-stimulating hormone (TSH), and the gonadotropins is regulated by releasing factors and in some instances by release of inhibitory factors by the hypothalamus. The concept of the regulatory role of the hypothalamus through neurotransmitters is generally accepted. Regulation of the release of the stimulatory or inhibitory factors by the hypothalamus involves complex neural circuitry in which the serotonergic and dopaminergic neurons represent links in the control mechanisms.[3] The hypothalamus has high concentrations of both these neurotransmitters—dopamine and serotonin—which are essentially antagonistic in their effects on pituitary hormone regulation.[14]

We have examined the hypothalamo–pituitary–thyroid relationship in pyridoxine deficiency. 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 (FIG. 1). Evidence suggests that TSH secretion is stimulated by serotonergic neurons. Injection of serotonin

TABLE 1. Pyridoxal Phosphate, Serotonin, Dopamine, and Noradrenaline Contents in Control and Pyridoxine-Deficient Rat Hypothalamus[a,b]

Animal Status	Pyridoxal Phosphate (nmol/g)	Serotonin (nmol/g)	Dopamine (nmol/g)	Noradrenaline (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[c]	1.00 ± 0.27[d]	1.25 ± 0.10	2.01 ± 0.10

[a]Values are means ± SEM of eight separate determinations in each group.
[b]From Dakshinamurti *et al.*[3] Used with permission.
[c]$p < 0.001$ compared with controls (Student's unpaired t-test).
[d]$p < 0.01$ compared with controls (Student's unpaired t-test).

into the third ventricle caused a rapid increase in serum TSH, an effect that was completely reversed by pretreatment of rats with the serotonin receptor antagonist cyproheptidine.[15] Although Krulich *et al.*[16] reported a decrease in serum TSH in rats after intraventricular injection of serotonin, Chen and Ramirez[17] indicate that serotonin stimulates TRH release from superfused hypothalamus. A direct relationship between hypothalamic serotonin turnover and TSH release has been reported by Smyth *et al.*[18] Dopaminergic neurons exert an inhibitory effect on the secretion of

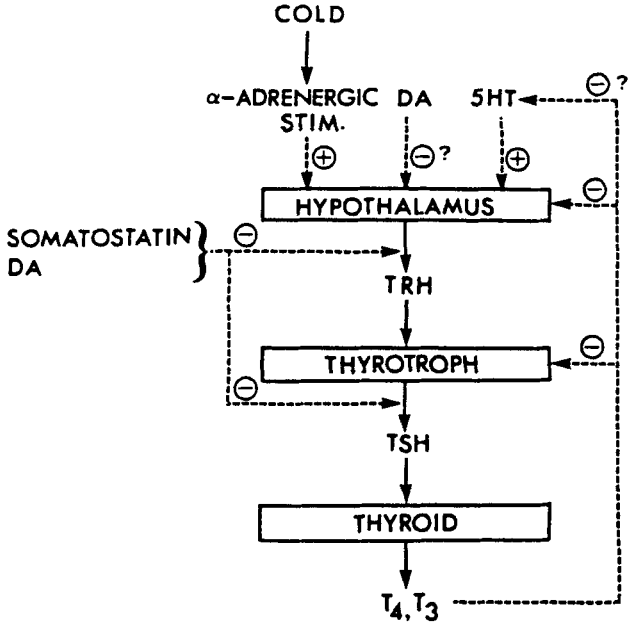

FIGURE 1. Hypothalamus–pituitary–thyroid relationship. (From Dakshinamurti *et al.*[3] Used with permission.)

TSH. This effect is at the level of the pituitary as bromocriptine blunts the stimulatory effect of TRH in euthyroid subjects.[19] The inhibitory effect of dopamine is abolished by dopamine receptor antagonists such as domperidone.[20] The cold-induced secretion of TSH is mediated by norepinephrine.[14] Studies using inhibitors of norepinephrine synthesis or α-adrenergic blockers have established a stimulatory role for norepinephrine in the control of TRH-mediated TSH secretion.[21] There is a difference between the α-adrenergic receptor subtypes, α_1 being inhibitory and α_2 being stimulatory.[22] On balance, it appears that serotonergic neurons have a stimulatory effect on hypothalamic control of pituitary secretion of TSH in situations where central control is natural, such as in timing of the circadian rhythm and, possibly, in the pulsatile secretion of TSH.[23]

The decrease in hypothalamic secretion with no change in its dopamine content led us to investigate the thyroid status of pyridoxine-deficient rats. These experiments were

TABLE 2. Serum Thyroxine (T_4), Tri-iodothyronine (T_3), TSH, and Pituitary TSH in Normal, Control, and Pyridoxine-Deficient Three-Week-Old Rats[a,b]

Treatment	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[c] (41)
T_3 (nmol/l)	1.54 ± 0.07 (20)	1.40 ± 0.06 (42)	0.98 ± 0.03[c] (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[c] (38)
Pituitary TSH (μg/pituitary)	2.12 ± 0.15 (17)	1.80 ± 0.12 (41)	1.05 ± 0.05[c] (38)

[a]Values are means ± SEM. Numbers of experiments are shown in parentheses.
[b]From Dakshinamurti *et al.*[26] Used with permission.
[c]$p < 0.01$ compared with group 1 and group 2, respectively (Duncan's multiple range test).
[d]$p < 0.05$ compared with group 1 (Duncan's multiple range test).

initially performed in three-week-old deficient, normal, and control groups of rats. Control rats were on a pyridoxine-supplemented diet and their food intake was adjusted so that they pair-weighted with deficient rats. Thyroxine (T_4) and tri-iodothyronine (T_3) concentrations in serum of the pyridoxine-deficient group was significantly lower than that of normal and control groups. Serum TSH concentration of the deficient group was not significantly different from normal and control groups. However, the pituitary TSH content of the deficient group was significantly lower than those of the other two groups (TABLE 2). These experiments were later repeated in appropriate groups of adult rats, yielding comparable results.[24] Pyridoxine treatment restored to normal the hypothalamic levels of pyridoxal phosphate and serotonin as well as the thyroid status of the animals.

The locus of the biochemical lesion leading to the hypothyroid state in pyridoxine deficiency was examined. The defect could be primary, at the level of the thyroid gland; secondary, with defective pituitary thyrotroph; or tertiary, with defective hypothalamic control. Primary hypothyroidism was ruled out as the low serum T_4 and T_3 values were not coupled with a compensatory rise in serum TSH. The classical formulation[25] of the hypothalamic–pituitary–thyroid axis would suggest pituitary hypothyroidism to be associated with a decrease in serum T_4 and T_3 coupled with a decrease in serum TSH and unresponsiveness to thyrotropin-releasing hormone (TRH). This was not compatible with our observations. Thyrotroph cells in pituitary gland from pyridoxine-deficient and control rats (FIG. 2A and B) were similar in their fine structural appearance characterized by well-developed organelles and the presence of numerous secretory granules. However, morphometric analysis of the numbers of secretory granules revealed a significant ($p < 0.05$) reduction in pyridoxine-deficient animals compared with controls.[26] The number of secretory granules per micrograph of thyrotroph examined were as follows (mean + SEM): control, 176 + 11; pyridoxine deficient, 142 + 12. Hypothalamic hypothyroidism is due to deficient TRH secretion. In examining this possibility we[27] injected TRH (15 μg/100 g body weight, i.p., every day for 7 days) into 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 pyridixone-deficient and control rats (TABLE 3). A significant increase in serum T_3 after TRH treatment was observed in pyridoxine-deficient rats but not in pyridoxine-supplemented controls. The experiments where deficient and control rats received thyroxine were designed to examine the integrity of the feedback regulation of TSH secretion by high levels of circulating T_4. Thyroxine treatment significantly decreased serum TSH in both pyridoxine-deficient and control rats pointing to the integrity of the pituitary control in both groups.

We also studied the kinetics of ligand binding using [^{3}H]methylhistidine analogue of TRH in membrane preparations from pyridoxine-deficient and control pituitaries; Scatchard analysis of the binding data indicated (TABLE 4) an increase in the number of receptors with no change in receptor affinity in the deficient pituitary membrane preparation.[27] These results are consistent with a hypothalamic type of hypothyroidism in pyridoxine-deficient rats caused by the specific decrease in hypothalamic serotonin.

The hypothalamus and brain neurotransmitters have a regulatory role on the secretion by the anterior pituitary of ACTH, growth hormone (GH), prolactin (PRL), and the gonadotropins. Developmentally the increase of adrenocortical response to stress coincides with the appearance of stress-induced changes in brain 5-HT metabolism.[28] Williams *et al.*[29] have shown that suprachiasmatic nucleus serotonergic terminals may play an important role in the synchronization of the circadian variation of corticosterone secretion. Yehuda and Meyer[30] have suggested that the pituitary–adrenal response to insulin-induced hypoglycemia was mediated by serotonin. Smythe *et al.*[31] reported that hypothalamic serotonergic activity was directly related to GH secretory status in the rat and that the serotonergic pathway participated in the GH negative-feedback effect in the hypothalamus. There seem to be sex-related variations in the kinetics of ligand binding to 5-HT receptors.[32]

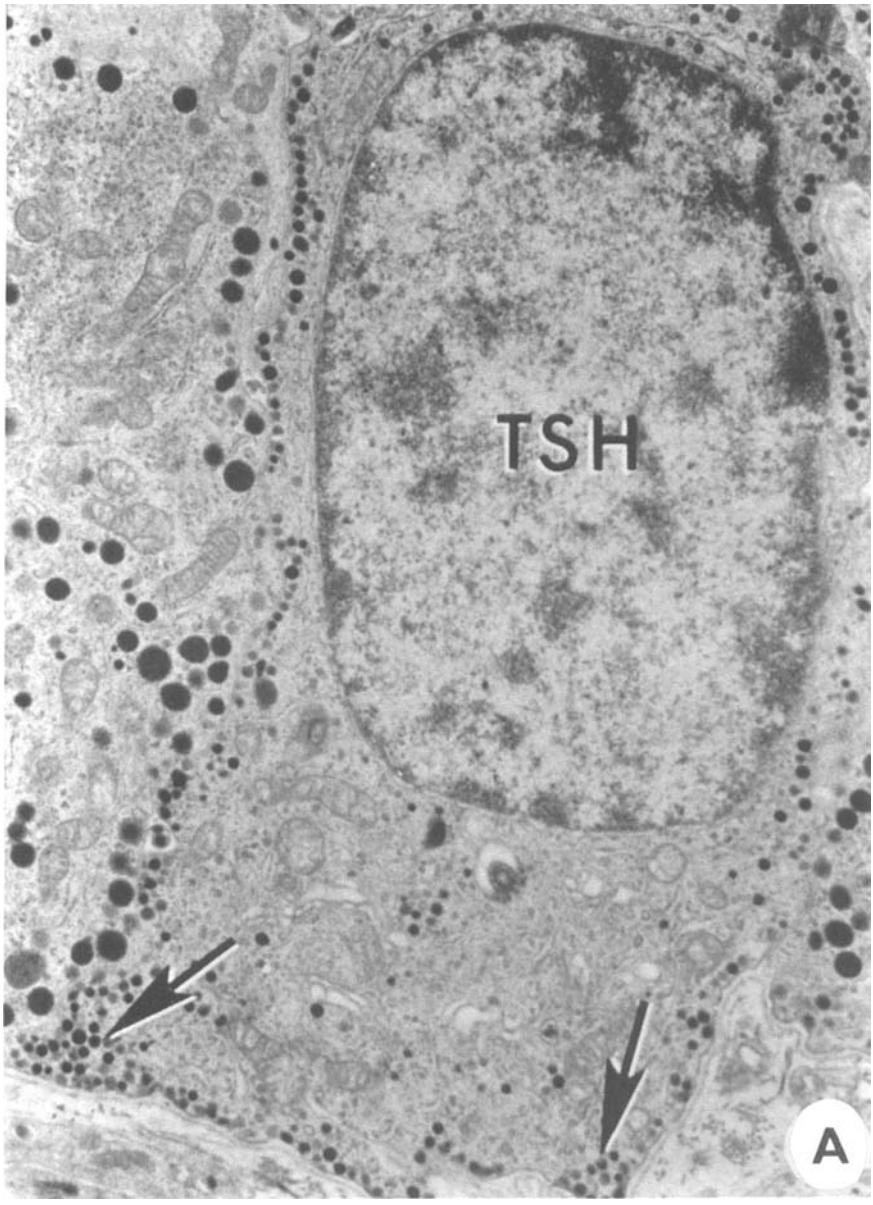

FIGURE 2A. Electron micrograph of a thyrotroph cell from control animal. Note moderate number of secretory granules (arrows). Magnification ×6000.

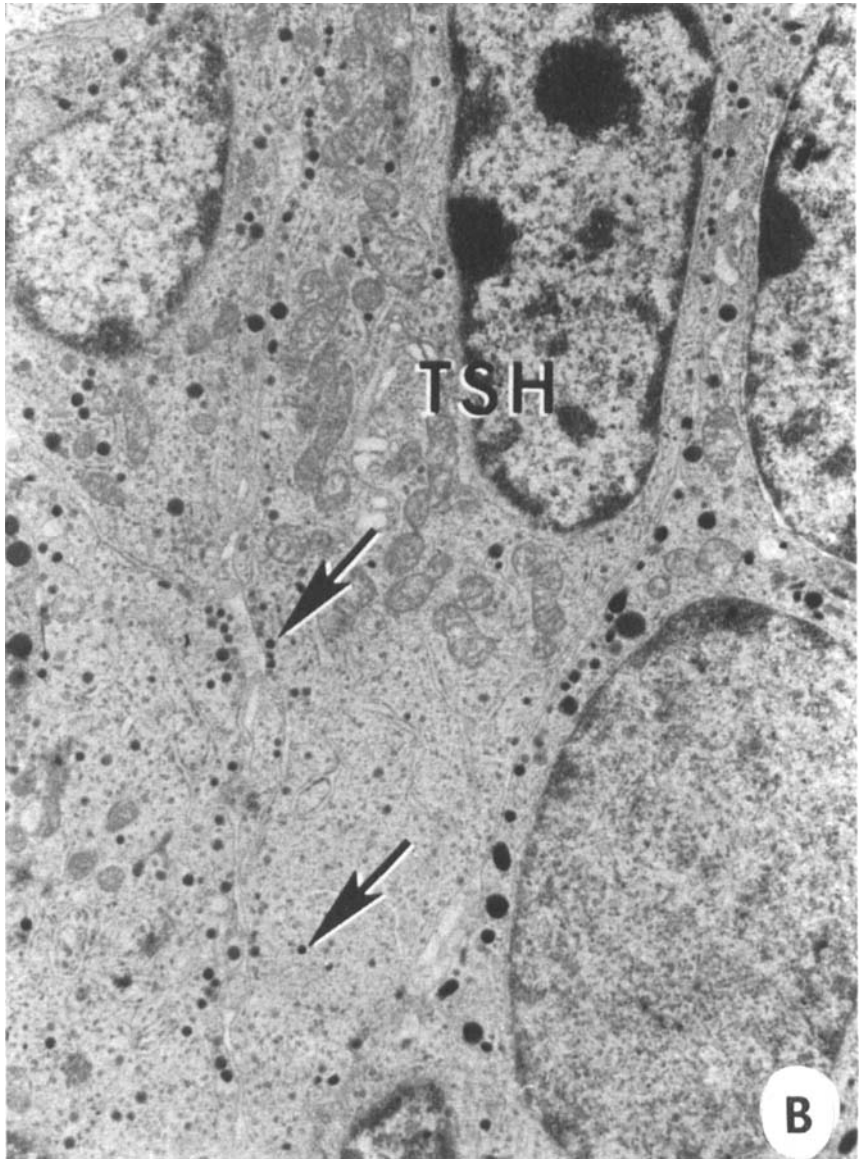

FIGURE 2B. Electron micrograph depicting reduction of secretory granules (arrows) in a thyrotroph cell from a pyridoxine-deficient animal. Magnification ×6000. (From Dakshinamurti *et al.*[26] Used with permission.)

TABLE 3. Effects of TRH and Thyroxine (T_4) on Pituitary TSH and Serum TSH, T_4 and Tri-iodothyronine (T_3) in Pyridoxine-Supplemented and Pyridoxine-Deficient Three-Week-Old-Rats[a,b]

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					
Pyridoxine-supplemented	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)
Pyridoxine-deficient	4.12 ± 10.15** (8)	0.81 ± 0.05** (8)	1.92 ± 0.23 (13)	52.00 ± 2.95* (16)	0.96 ± 0.05* (16)
TRH					
Pyridoxine-supplemented	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)
Pyridoxine-deficient	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					
Pyridoxine-supplemented	1.28 ± 0.08†† (7)	0.20 ± 0.02†† (7)	1.13 ± 0.05† (7)	901 ± 29†† (7)	24.86 ± 1.22†† (7)
Pyridoxine-deficient	3.76 ± 0.34** (5)	0.62 ± 0.05**† (5)	1.23 ± 0.13† (6)	1330 ± 104**†† (5)	21.60 ± 1.03**†† (5)

[a] Values are means ± SEM. Numbers of experiments are shown in parentheses. *$p < 0.05$ and **$p < 0.01$ compared with pyridoxine-supplemented group.† $p < 0.05$ and ††$p < 0.01$ compared with saline-treated group (Duncan's multiple range test).

[b] From Dakshinamurti *et al.*[27] Used with permission.

The activation of the serotonergic system stimulates secretion of PRL.[33] Direct stimulation of 5-HT receptor by injection of 5-HT into the third ventricle[34] or by administration of receptor stimulant quipazine[35] results in an increase of serum PRL. Melatonin has been shown act at the level of the hypothalamus, possibly acting via the serotonergic pathway, to increase PRL release.[36] PRL release from anterior pituitary is inhibited by dopamine,[37] which is generally considered to be a PRL release inhibitory factor. The administration of large doses of pyridoxine has been reported to reduce the PRL surge[38] resulting in the suggestion that pyridoxine is a dopamine agonist. This does not take into account the role of pyridoxine in the formation of both DA and

TABLE 4. [^{3}H](3-Methyl-Histidine^2) TRH ([^{3}H]MeTRH) Binding in the Pituitary of Three-Week-Old Rats[a,b]

Animal Status	[^{3}H]MeTRH Binding[c]	
	B_{max}(fmol/mg protein)	K_d(nmol/l)
Normal	141.40 ± 3.34	1.78 ± 0.25
Pyridoxine-supplemented (control)	144.38 ± 6.15	2.22 ± 0.49
Pyridoxine-deficient	181.20 ± 7.39[d]	2.38 ± 0.31

[a] Values are means ± SEM of eight separate determinations in each group.

[b] From Dakshinamurti *et al.*[27] Used with permission.

[c] B_{max}, maximal binding; K_d, dissociation constant.

[d] $p < 0.005$ compared with control (Student's unpaired *t*-test).

5-HT. However, under conditions of mild pyridoxine deficiency, the PRL release inhibitory effect would dominate.

PINEAL AND MELATONIN SECRETION

The pineal gland transduces photoperiodic information and hence has a crucial role in the temporal organization of various metabolic, physiological, and behavioral processes. Melatonin is the major secretory product of the pineal gland. The initial precursor for melatonin is tryptophan, which is hydroxylated in the pinealocyte to 5-HTP and decarboxylated to yield serotonin. The highest concentration of serotonin in the body is in the pineal gland where it is converted to *N*-acetylserotonin (NAS) by the enzyme *N*-acetyltransferase. NAS is converted to melatonin by hydroxyindole-*O*-methyltransferase. Melatonin synthesis is stimulated by β-adrenergic postganglionic sympathetic fibers from the superior cervical ganglion which are stimulated in the dark. Melatonin levels in tissues and body fluids show both circadian and seasonal rhythms.

We have examined[39] the effect of a moderate deficiency of pyridoxine on indolamine metabolism in the pineal gland of adult male rats. Melatonin and NAS showed significant circadian variation in both pyridoxine-deficient and control animals (FIG. 3). However, the peak nighttime levels of pineal melatonin and NAS were significantly lower in pyridoxine-deficient animals. Pineal 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) levels were significantly lower in pyridoxine-deficient animals.

Isoproterenol administration stimulated the synthesis of pineal NAS and melatonin in both control and pyridoxine-deficient rats. However, the increases in NAS and melatonin were significantly lower in the deficient rats. Treatment of pyridoxine-deficient rats with pyridoxine restored the levels of pineal 5-HT, NAS, and melatonin to values seen in pyridoxine-supplemented control animals (TABLE 5). Such a reversal of the effects of pyridoxine deficiency was evident both during day and night periods. There was no significant difference in pineal *N*-acetyltransferase (NAT) between control and pyridoxine-deficient rats. However, pineal 5-HTP decarboxylase activity was significantly decreased in pyridoxine-deficient rats (TABLE 6). Increase in the level of 5-HTP along with the decreased activity of 5-HTP decarboxylase in pineal glands of pyridoxine-deficient rats indicates that the formation of 5-HT from 5-HTP is significantly decreased in pyridoxine-deficient animals.

Tryptophan hydroxylation is considered to be the rate-limiting step in the synthesis of serotonin.[40] Several studies have indicated that a decrease in pineal 5-HT can reduce melatonin synthesis. *In vivo* administration of inhibitors of an aromatic amino acid decarboxylase, such as benzerazide[40] or monofluoromethyl dopa,[41] results in a reduction in the synthesis of pineal 5-HT and melatonin levels without altering pineal NAT activity. Our results demonstrate that pyridoxine deficiency in adult rats caused significant decreases in pineal NAS and melatonin synthesis due to decreased decarboxylation of 5-HTP. Thus, 5-HT availability, in addition to other known factors, could be important in the regulation of the synthesis of melatonin. The best understood endocrinological function of the pineal is the antigonadotropic action of melatonin.[42] Other studies indicate that melatonin acts at the level of the hypothalamus, affecting the formation of releasing factors for anterior pituitary hormones.[36] It has been

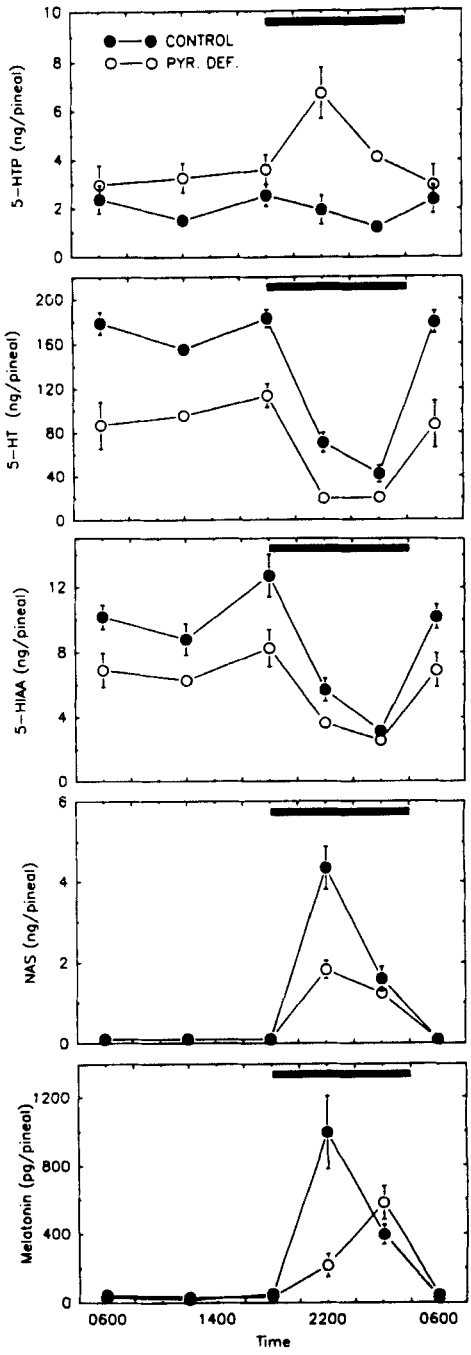

FIGURE 3. Effect of pyridoxine deficiency on the circadian variation in rat pineal 5-HTP, 5-HT, 5-HIAA, NAS, and melatonin. Black bar indicates duration of darkness. Values are mean + SEM. (From Viswanathan *et al.*[39] Used with permission.)

TABLE 5. Effect of Pyridoxine Administration on Pineal Content of 5-HT, 5-HIAA, NAS, and Melatonin[a]

	5-HT	5-HIAA	NAS	Melatonin
Day (12.00 hr)				
Control	155.08 ± 5.0	8.77 ± 1.0	N.D.	0.02 ± 0.003
Pyridoxine-deficient	95.17 ± 6.7[b]	6.25 ± 0.3[b]	N.D.	0.03 ± 0.01
Control + pyridoxine	164.54 ± 5.8	12.47 ± 1.2	N.D.	0.02 ± 0.004
Pyridoxine-deficient + pyridoxine	169.95 ± 3.0	13.12 ± 0.8	N.D.	0.02 ± 0.01
Night (22.00 hr)				
Control	70.33 ± 9.0	5.69 ± 0.7	4.34 ± 0.5	0.67 ± 0.07
Pyridoxine-deficient	19.61 ± 1.7[b]	3.65 ± 0.2[b]	1.83 ± 0.2[b]	0.21 ± 0.07[b]
Control + pyridoxine	55.21 ± 9.8	5.89 ± 0.6	4.56 ± 0.8	0.48 ± 0.09
Pyridoxine-deficient + pyridoxine	76.52 ± 15.3	5.95 ± 0.7	4.20 ± 0.4	0.53 ± 0.09

[a]From Viswanathan *et al.*[39] Used with permission.
[b]$p < 0.05$ compared to the control animals. Values in ng/pineal are mean ± SEM (N.D. = not detected).

suggested that melatonin might act through the serotonergic pathway,[43] although direct effects of melatonin on pituitary, adrenals, and thyroid are indicated.[42]

SEIZURE ACTIVITY

In early experiments[44] dietary deficiency of pyridoxine was induced in dams during the last week of gestation. The pups had congenital deficiency of pyridoxine, assessed biochemically. Among the litters of deficient mothers, spontaneous convulsions were seen around 3–4 days of age. The fits were characterized by high-pitched screams, followed by generalized convulsions of a few seconds' duration repeated many times during the day. The sequences of frames at intervals of 0.6 and 0.3 seconds, respectively, from a motion picture of the pups (FIG. 4) shows the pups on the lower right of the frame undergoing convulsions. The brain levels of pyridoxal phosphate and glutamic acid decarboxylase were significantly decreased in these animals.

In later work[45] pyridoxine deficiency was induced in dams during lactation and the pups used in experiments between three to five weeks of chronological age. Uni- and

TABLE 6. Effect of Pyridoxine Deficiency on Rat Pineal 5-HTP Decarboxylase and NAT Activities[a,b]

Experimental Group	5-HTP Decarboxylase Activity	NAT Activity
Control	19.37 ± 0.79	12.80 ± 5.2
Pyridoxine-deficient	6.88 ± 0.77[c]	13.04 ± 3.1

[a]Values represent mean ± SEM ($n = 6$). Enzyme activities are expressed in nmol/pineal per hour.
[b]From Viswanathan *et al.*[39] Used with permission.
[c]$p < 0.001$ compared to control by Student's t-test.

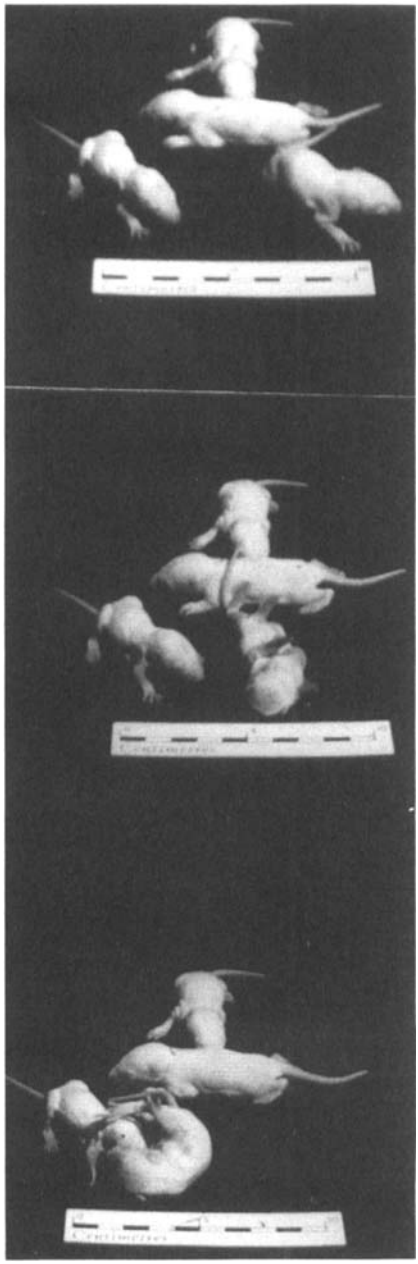

FIGURE 4. Convulsions seen in pyridoxine-deficient neonatal rat at (**a**) zero time; (**b**) 0.6 sec. after (a); (**c**) 0.3 sec after (b). (From Dakshinamurti and Stephens.[44] Used with permission.)

bipolar EEG recordings were taken while the animals were under the influence of Nembutal anesthesia. In contrast to slow-wave EEG activity in control rats, pyridoxine-deficient rats exhibited high-voltage spikes and wave discharges (FIG. 5). Pyridoxal phosphate and γ-amino-butyric acid (GABA) levels in the cerebellum of these animals were significantly decreased when compared with appropriate controls. The high-affinity binding of triatiated GABA to $GABA_a$ receptors and of tritiated baclofen to $GABA_b$ receptors was studied in synaptosomal membrane preparations prepared from the cerebellum of pyridoxine-deficient and appropriate control rats.[11] There was a significant increase in the maximal binding of ligands to both $GABA_a$ and $GABA_b$ receptors with no difference in their binding affinities (TABLE 7). The changes observed

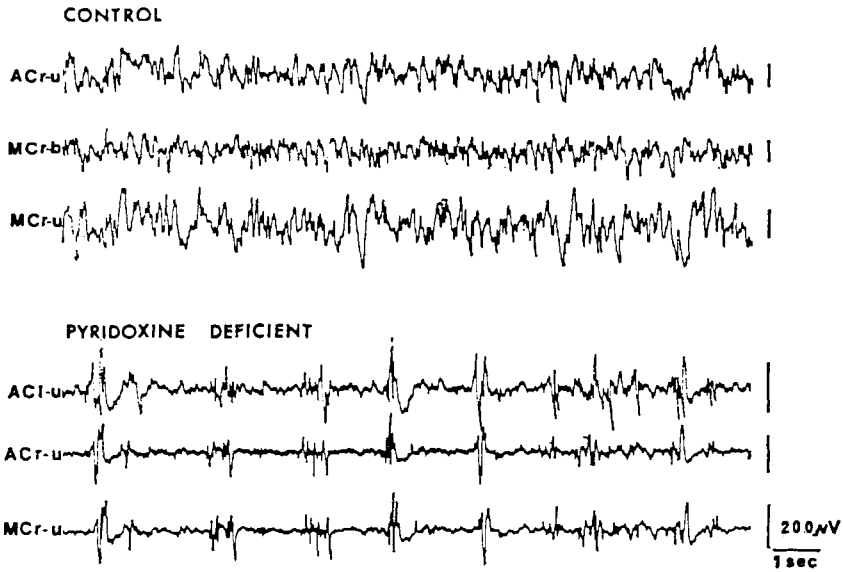

FIGURE 5. EEG of a control (upper curves) and a pyridoxine-deficient rat (lower curves) taken with unipolar and bipolar recordings, under light Nembutal anesthesia. AC, auditory cortex; MC, motor cortex; l, left hemisphere; r, right hemisphere; U, unipolar; and b, bipolar recordings, respectively. (From Stephens *et al.*[45] Used with permission.)

suggest a supersensitivity of these receptors due to chronic decrease in the synaptic release of GABA.

In more recent work we used adult male rats (150 g body weight) to induce dietary pyridoxine deficiency. These rats were moderately pyridoxine deficient, assessed biochemically. We have studied picrotoxin-induced seizure activity in deficient and control rats. Picrotoxin, a $GABA_a$ receptor antagonist, was injected (30–100 ng/μl) steriotaxically in thalamic ventro-posterior-lateral (VPL) nucleus. VPL unit discharge activity and parieto-occipital EEG were monitored to assess threshold, duration, and severity of seizures as well as recovery time following intraperitoneal or intracerebral administration of pyridoxine or intracerebral administration of GABA. Pyridoxine-

TABLE 7. Pyridoxal Phosphate and γ-Aminobutyric Acid (GABA) Concentrations in the Cerebellum of Three-Week-Old Rats[a,b]

Experimental Group	Pyridoxal Phosphate (ng/g wet wt)	GABA (μmoles/g wet wt)
Control	1056 ± 54	2.62 ± 0.42
Pyridoxine-deficient	568 ± 72[c]	1.15 ± 0.32[d]

[a]Mean ± SEM were determined from six to eight separate experiments, each assayed in triplicate.
[b]From Paulose and Dakshinamurti.[12] Used with permission of publisher.
[c]$p < 0.001$ with respect to control.
[d]$p < 0.01$ with respect to control.

deficient animals had a reduced threshold for picrotoxin-induced seizure activity. At any given dose of picrotoxin, deficient rats exhibited increased severity and duration of seizure activity. Contralateral microinjections of picrotoxin significantly suppressed thalamic (VPL) unit activity and induced burst discharge activity in a dose- and time-dependent manner (FIG. 6A). Thalamic (VPL) burst discharge activity was functionally correlated with the parieto-occipital EEG spike and wave activity in normal and pyridoxine-deficient rats. Microinjections of either GABA or pyridoxine in the thalamic (VPL) region significantly suppressed the burst discharge activity and

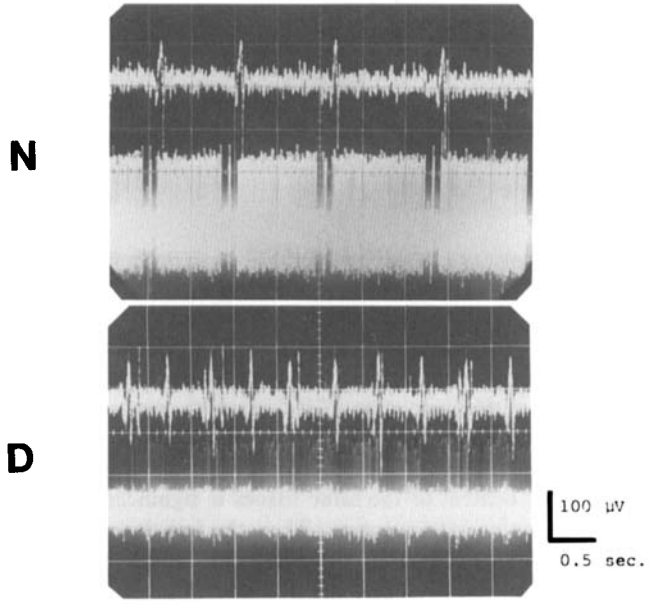

FIGURE 6A. Picrotoxin (30 ng) induced thalamic burst discharge activity, exhibiting a functional relationship with parieto-occipital EEG spike and wave activity (upper panel) in normal (N) and pyridoxine-deficient (D) rat. EEG spike and wave activity was more pronounced in pyridoxine-deficient animals.

parieto-occipital EEG spike and wave activity. This suppression was dose and time dependent. Injections of either GABA or pyridoxine at the sites located above the thalamus did not suppress electrical seizure discharge activity. Recovery following pyridoxine administration took longer in the deficient rats (FIG. 6B). GABA-induced neuronal recovery was faster in both the pyridoxine-deficient and control rats due to its direct inhibitory action. The slower pyridoxine-induced recovery might be routed through pyridoxal phosphate (PLP)-dependent mechanisms, including the formation of GABA.

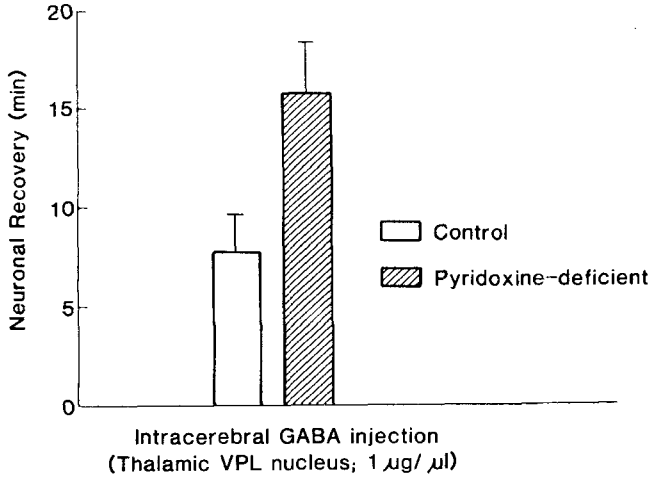

FIGURE 6B. GABA-induced neuronal recovery from picrotoxin-epileptogenesis was significantly delayed in pyridoxine-deficient animals ($p < 0.05$).

CONCLUSIONS

In summary, pyridoxal phosphate-dependent decarboxylases have a very important role in the synthesis of putative neurotransmitters. These decarboxylases differ from each other in the affinity of the respective apoenzyme for pyridoxal phosphate. Thus, in a partial deficiency of pyridoxine, some PLP-dependent enzymes would be affected more than others leading to a greater depletion of some neurotransmitters. There is no decrease in the dopamine content of several brain areas in the pyridoxine-deficient rat. However, the serotonin content of the same tissues is significantly and functionally decreased in the pyridoxine-deficient state. Thus, the imbalance between dopamine and serotonin in the hypothalamus of the pyridoxine-deficient rat leads to severe neuroendocrine consequences. Similarly the decrease in pineal serotonin leads to a deficiency of melatonin and its sequela. The decrease in cerebral and cerebellar GABA content in the pyridoxine-deficient rat is accompanied by a significant increase in the concentration of the excitatory amino acid, glutamic acid. Spontaneous or drug-

induced seizure activity in the pyridoxine-deficient rat is ascribed to this neurotransmitter imbalance.

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