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NEUROBIOLOGY OF PYRIDOXINE

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Pyridoxal 5'-phosphate (PLP) is the major coenzymatic form of pyridoxine. There are over one hundred known pyridoxal 5'-phosphate-dependent reactions, most of which are involved in the metabolism of various amino acids. Pyridoxamine 5'-phosphate can function in aminotransferase reactions by the cyclic regeneration of the two active phosphate forms. Pyridoxal 5'-phosphate-dependent reactions studied in the nervous system are involved in the catabolism of various amino acids. The putative neurotransmitters, dopamine, norepinephrine, serotonin, histamine, γ-aminobutyric acid and taurine, as well as the sphingolipids and polyamines are synthesized by PLP-dependent enzymes. Of these enzymes, three (glutamic acid decarboxylase, 5-hydroxytryptophan decarboxylase and ornithine decarboxylase) seem to have crucial roles (Fig. 1). The clinical effects of pyridoxine deficiency can be explained on the basis of the known decreases in the activities of these enzymes (1).

EXPERIMENTAL PYRIDOXINE DEFICIENCY IN THE NEONATE RAT

Dakshinamurti and Stephens (2) first reported the production of congenital pyridoxine deficiency. Our observations extended the "chronic fetal distress" hypothesis of Gruenwald (3) to include effects on the development of the central nervous system. We later showed that a deficiency of pyridoxine in rat pups could be produced by depriving the dam of dietary pyridoxine during lactation (4). Such a deficiency has been characterized using biochemical and electrophysiological parameters. The electroencephalogram (EEG)

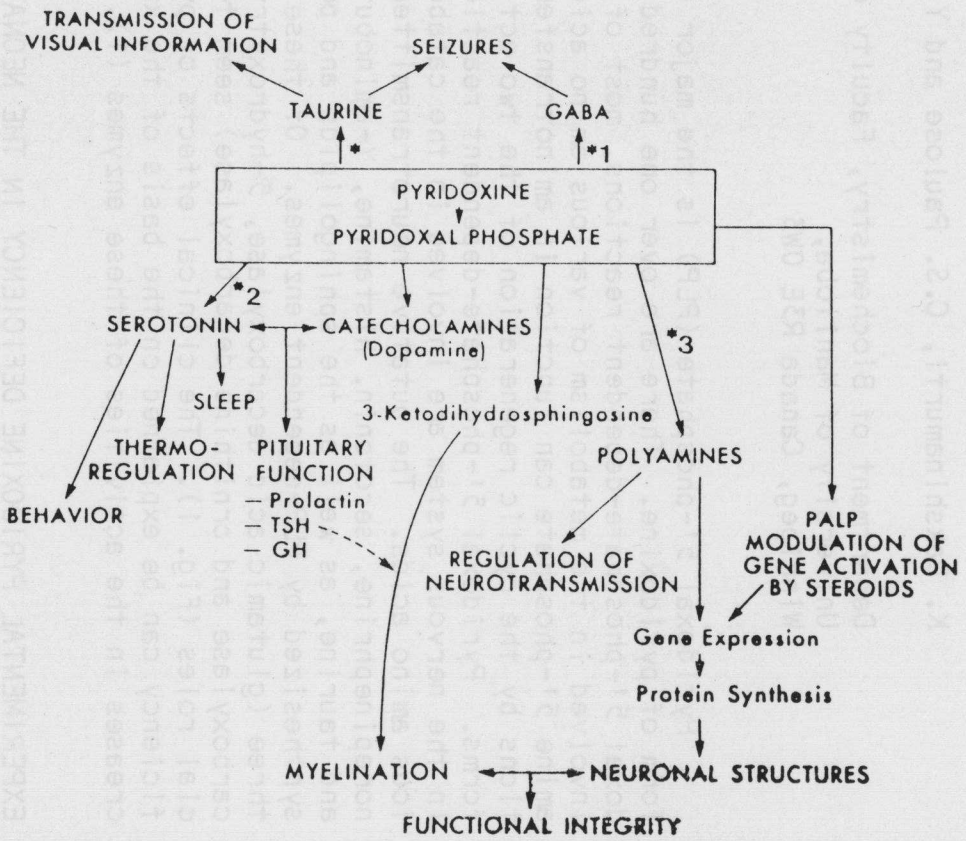

Fig. 1. Involvement of pyridoxine in the central nervous system (CNS).

of pyridoxine-deficient animals showed spike activity, presumably indicative of the existence of seizures in many of the deficient rats (Fig. 2). In a study of the oxidative reactions, there was no difference between mitochondria prepared from brains of pyridoxine-deficient and pyridoxine-supplemented neonates in terms of oxygen consumption, ADP/O and respiratory carriers (5). Rats fed the pyridoxine-deficient diet exhibited significantly lower levels of PLP and γ-aminobutyric acid (GABA). The activities of glutamic acid decarboxylase (GAD) in the presence or absence of exogenously added PLP reflected the lack of availability of PLP in the deficient state (Table 1). The activity of GABA-transaminase (GABA-T) was not different between pyridoxine-deficient and pyridoxine-supplemented rat brains. This is ascribed to the greater affinity of GABA-T for PLP compared with GAD. The bursts of high voltage spikes during spontaneous EEG activity reflect the decrease in cerebral GABA in the deficient rats. Evoked potentials presented abnormalities in their latency, wave form and response to repetitive stimuli and the extent to which they were affected depended on the intensity of the deficiency. The changes observed in the auditory evoked potentials in the deficient rats were the result of retardation of normal ontogenetic development of the central nervous system (CNS) of these animals.

TAURINE

The distribution of taurine in the nervous system is heterogeneous with high levels in the synaptic vesicles (6). With the exception of kittens and the human infant, growing animals of most species have the capacity to synthesize taurine (7). Regardless of the synthetic pathway used, the crucial decarboxylation reaction is catalyzed by a PLP-dependent decarboxylase. In view of the demonstrated inhibitory effect of taurine on neuronal activity (8-10) and its role in the visual system (11), we determined the cysteine sulfinic acid decarboxylase activity in whole brain homogenates of rat pups deficient in taurine or taurine and pyridoxine. The results (Table 2) indicate a significant reduction in both glutamic acid decarboxylase and cysteine sulfinic acid decarboxylase, due specifically to the decrease in tissue PLP. It is significant that very high concentrations of both apoenzymes are observed in the deficient rat brain.

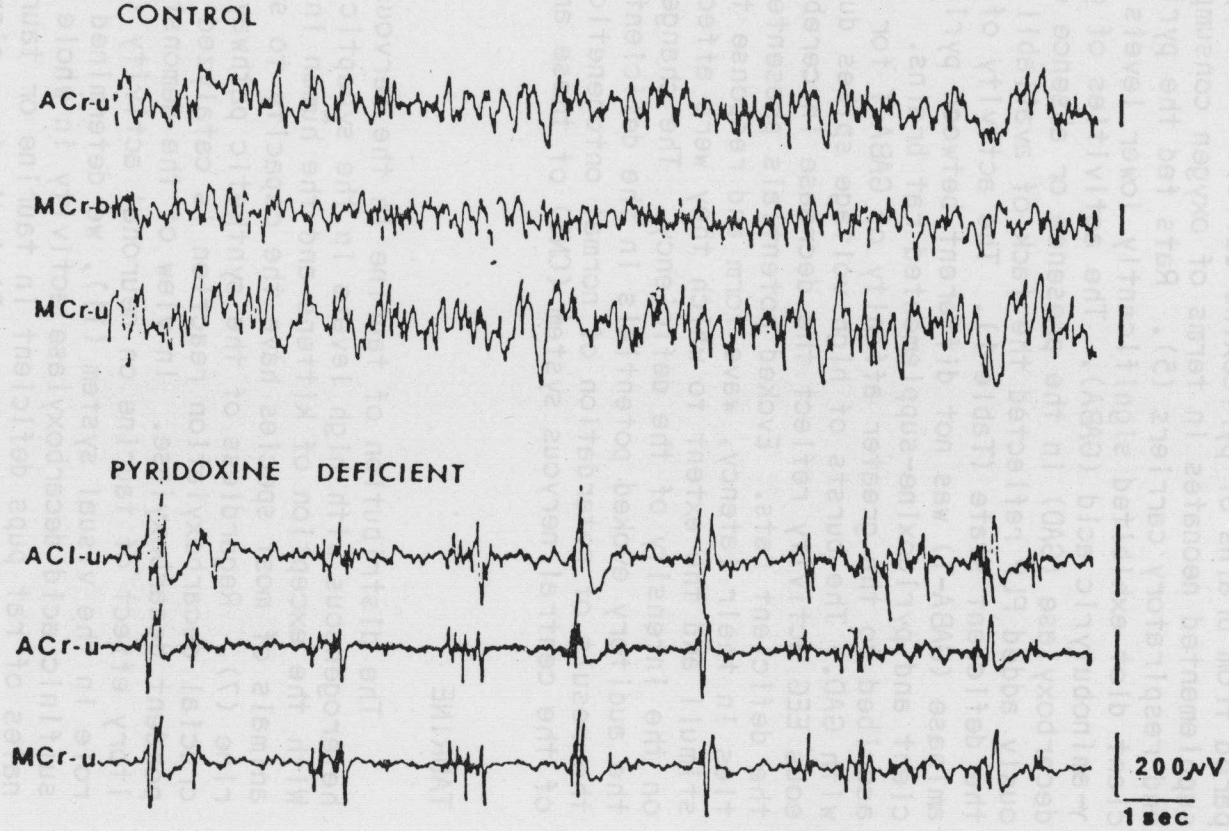

Fig. 2. EEG of a control (upper curves) and a pyridoxine-deficient rat (lower curves), taken with unipolar and bipolar recordings, under light Nembutal anaesthesia. AC, auditory cortex; MC, motor cortex; l, left hemisphere; r, right hemisphere; u, unipolar and b, bipolar recordings, respectively.

Table 1. Glutamate Decarboxylase (GAD) and Cysteine Sulfinic Acid Decarboxylase (CSAD) Activities in Whole Brain of 3-Week Old Rats

Experimental Group	GAD Activity μ mol/hr/mg protein		CSAD Activity μ mol/hr/mg protein	
	With Added PLP[a]	No Added PLP	With Added PLP[a]	No Added PLP
Control (Group 1)	4.76 ± 0.32	0.92 ± 0.09	7.89 ± 0.95	2.27 ± 0.30
Taurine-Deficient (Group 2)	6.41 ± 0.20[c]	0.84 ± 0.11	9.67 ± 1.32	2.16 ± 0.33
Taurine + Pyridoxine-Deficient (Group 3)	15.55 ± 0.75[b]	0.41 ± 0.07[b]	21.45 ± 3.76[b]	0.88 ± 0.33[b]

Values represent mean ± S.E.M. of 5 separate experiments, each performed in triplicate.
[a] 0.2 mM pyridoxal 5'-phosphate (PLP)
[b] $P<0.01$ compared with group 1 and group 2, respectively
[c] $P<0.05$ compared with group 1 (Duncan's multiple range test).

Table 2. Whole Brain Levels of Pyridoxal 5'-Phosphate, GABA, GAD and GABA-T in 5-6-Week Old Pyridoxine-Deficient and Control Rats

Pyridoxine Status	PLP Content (nmoles/g fresh wt.)	GABA (μmoles/g fresh wt.)	GAD Activity (munits[a]/g protein)		GABA-T (unit[b]/ g protein)
			no added PLP	added PLP	
Pyridoxine-supplemented (ad lib fed)	3.64 ± 0.26 (7)	1.53 ± 0.05 (8)	340 ± 50 (9)	3790 ± 140 (9)	51.7 ± 16.0 (4)
Pyridoxine-supplemented (restricted food, pair-weighed)	3.52 ± 0.42 (4)	1.38 ± 0.06 (4)	478 ± 28 (4)	2842 ± 415 (4)	37.8 ± 3.5 (3)
Pyridoxine-deficient	2.54 ± 0.34[c] (7)	0.64 ± 0.06[d] (4)	140 ± 30[c] (7)	5920 ± 390[e] (7)	36.9 ± 2.0 (5)

Values represent mean ± S.E.M. for the number of animals indicated in paretheses.
[a]One enzyme unit decarboxylates 1 mole of L-glutamic acid per minute.
[b]One enzyme unit transaminates 1 mole of GABA per 30 minutes.
[c]$P<0.025$, [d]$P<0.005$, [e]$P<0.001$ with respect to both ad lib fed and pair-weighed controls.

MYELINATION

Although a role for PLP as a cofactor in one of the steps leading to the synthesis of sphingosine was known early (12), it was only much later that the consequences of pyridoxine deficiency during the critical period of development in the rat on cerebral lipids were investigated. The incorporation of [^{14}C]acetate into all major lipid classes in brain was significantly decreased (13). The specific radioactivities of purified cerebrosides and sulfatides from pyridoxine-deficient rat brain were only one-fifth those of pyridoxine-supplemented controls (14). The fatty acid composition of brain galactolipids were examined. An accumulation of stearic acid and correspondingly, a significant decrease in the contents of lignoceric and nervonic acids were found (15,16). This was related to a defect in the long chain fatty acid elongation system in pyridoxine deficient rat brain, which was similar to that seen in hypothyroidism (17,18). Thus, the CNS myelin of pyridoxine-deficient rats is qualitatively and quantitatively different from that in pyridoxine-supplemented controls. The specific activity of 2',3'-cyclic nucleotide 3'-phosphohydrolase, a marker enzyme for myelin, was decreased in pyridoxine-deficient neonatal rat brain (19).

MONOAMINES

Decarboxylation of the precursor amino acid is a necessary step in the formation of the putative neurotransmitters such as dopamine, norepinephrine, 5-hydroxytryptamine (serotonin), γ-aminobutyric acid (GABA) and taurine. As PLP is the coenzyme of these decarboxylases, we determined the concentration of various neurotransmitters in the pyridoxine-deficient rat brain. There was no change in the steady-state concentration of dopamine and norepinephrine in pyridoxine-deficient rat brain (20), an observation in striking contrast to that of Shoemaker and Wurtman (21) who found significant decrease in brain catecholamines of rats subjected to undernutrition perinatally. In experiments where the further metabolism of monoamines was reduced by treatment of the animals with the monoamine oxidase inhibitor pargyline, we found (20) that the concentration of brain 5-hydroxytryptamine (serotonin) in pyridoxine-deficient rats was significantly reduced (Table 3). The possibility that this decrease resulted from inanition and the resultant mal-

nutrition was ruled out by using pair-weighed pyridoxine-supplemented controls. The level of brain tryptophan was not affected by dietary deficiency. The activity of brain tryptophan hydroxylase was not decreased in deficiency. Increased catabolism of serotonin or transport to cerebrospinal fluid were ruled out by appropriate control experiments. Our observation of non-parallel changes in brain concentrations of catecholamines and serotonin, respectively, would indicate the syntheses of the various monoamines are regulated separately.

Table 3. Effect of Pargyline Treatment of Brain Monoamines

	B-6 Deficient	B-6 Supplemented
5-HT (nmoles/g)		
Untreated	1.59 ± 0.07[a]	2.15 ± 0.08
Treated	3.34 ± 0.11[b]	7.31 ± 0.28
NE (nmoles/g)		
Untreated	2.14 ± 0.14	2.46 ± 0.08
Treated	3.91 ± 0.06	3.56 ± 0.14
DA (nmoles/g)		
Untreated	2.92 ± 0.24	3.15 ± 0.05
Treated	4.09 ± 0.06	4.45 ± 0.22

Values are means ± S.D. of 8 rats in each group.
[a]$P<0.01$ and [b]$P<0.005$ with respect to +B-6 controls.
5-HT, 5-hydroxytryptamine; NE, norepinephrine; DA, dopamine.

The enzyme aromatic amino acid decarboxylase has been reported (22) to be capable of decarboxylating a variety of amino acids including dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan (5HTP). Immunological evidence has been presented to suggest the identity of DOPA decarboxylase with

5HTP decarboxylase (23). Their data indicate that ten times more antibody, prepared against the hog kidney decarboxylase, per unit of enzyme activity was required for the complete inhibition of the decarboxylase activity of rat brain as compared to the enzyme activity of hog kidney. Other reports (24) have suggested that the optimal conditions for the decarboxylations of DOPA and 5HTP, respectively, were different.

In examining the decarboxylations of DOPA and 5HTP by brain homogenates using sensitive methods, we found that the pH optima for these two reactions are distinct (Fig. 3). The activity ratios of DOPA decarboxylase/5HTP decarboxylase measured under optimal substrate and cofactor concentrations were different in the various brain areas studied. In pyridoxine deficiency, there were no parallel decreases of DOPA and 5HTP decarboxylase activities in various brain regions (Table 4). Dialysis of brain homogenates, in the

Table 4. Distribution of DOPA Decarboxylase and 5HTP Decarboxylase Activities in Pyridoxine-Deficient Rat Brain Regions Relative to that in Pyridoxine-Supplemented (Normal) Rats

Brain Regions	Maximal Enzyme Activity*		Holoenzyme Activity*	
	DOPA decarboxylase	5HTP decarboxylase	DOPA decarboxylase	5HTP decarboxylase
Cerebellum	53.7	34.5	23.7	0.0
Cerebral Cortex	32.6	47.0	36.7	0.0
Corpus Striatum	50.7	49.8	36.5	20.8
Hypothalamus	70.3	49.3	68.8	12.9

* Of deficient rat brain as percent of normals.

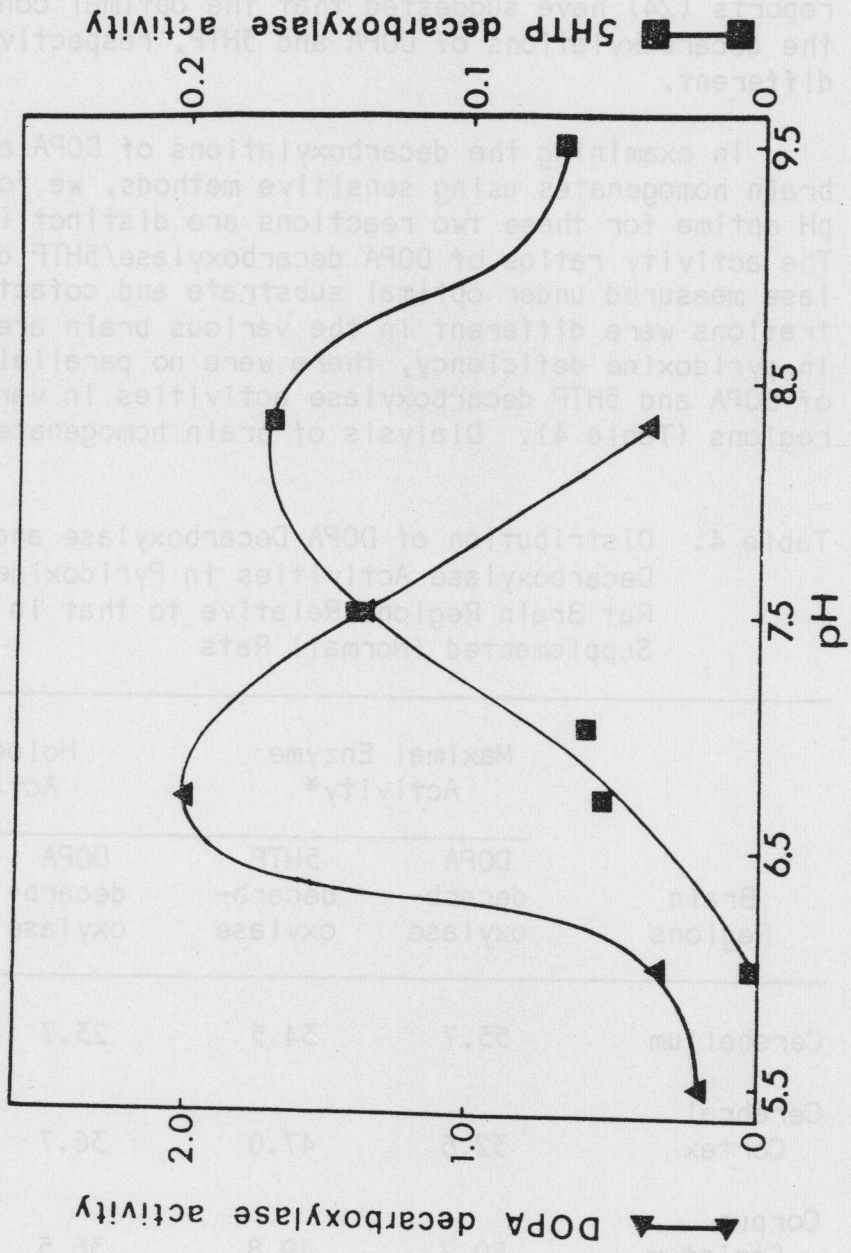

Fig. 3. The activities of brain DOPA decarboxylase and 5HTP decarboxylase at various pHs. Sodium phosphate buffer was used for assays at pH 5.5-7.5 while Tris-HCl buffer was used for assays at pH 7.5-9.5. Enzyme activities are expressed as nmol/min/mg protein.

presence or absence of hydroxylamine, resulted in a total or greater loss of 5HTP decarboxylase activity as compared to DOPA decarboxylase activity (Table 5). In addition to distinct pH optima, there seems to be differences in the affinity for PLP between DOPA and 5HTP decarboxylating enzymes. These results are consistent with the suggestion that the two enzyme activities are catalyzed by distinct protein species.

Table 5. Effect of Dialysis on Whole Brain DOPA Decarboxylase and 5-HTP Decarboxylase

	PLP added (mM)	DOPA decarboxylase activity %	5HTP decarboxylase activity %
Undialyzed whole brain homogenate	0	26	8
	0.025	89	-
	0.050	92	-
	0.125	100*	64
	0.300	-	100**
Homogenate dialyzed against buffer	0	31	0
	0.025	101	-
	0.050	92	-
	0.125	93	45
	0.300	-	103
Homogenate dialyzed against buffer containing hydroxylamine	0	26	0
	0.025	27	-
	0.050	36	-
	0.125	65	0
	0.150	-	14
	0.300	-	69

*Values represent the average of 3 separate experiments.
**Represents an activity of 1024 pmol/min/mg protein.
Represents an activity of 39.2 pmol/min/mg protein.

CONSEQUENCES OF DECREASED BRAIN SEROTONIN

The physiological implications of the significant decrease of brain serotonin in pyridoxine-deficient rats were explored. There was a consistent decrease in deep body temperature in the animals (20). Myers (25) has presented evidence to support the view that a serotonergic mechanism in the hypothalamus is involved in thermoregulation in the rat. Pyridoxine-deficient young rats also showed a significant decrease in their motility (20). Similar results in mice have been reported by Tunnicliff et al. (26). We also found that pyridoxine deficiency affected sleep in rats in two ways (1). The duration of deep slow-wave sleep 2 (SWS 2) is shortened and in some instances completely abolished. REM sleep is also affected in the same manner. These animals are in shallow slow-wave sleep (SWS 1). The effects of pyridoxine deficiency on sleep parallel the effects of experimental serotonin deficiency in animals and man, in keeping with the hypothesis of Jouvet (27) that serotonergic neurons play a major role in maintenance of slow-wave sleep 2 and REM (paradoxical sleep) events. This is confirmed by the work of Kilanmaa and Fuxe (28) who injected 5,7-dihydroxytryptamine bilaterally into rat dorsomedial mesencephalic tegmentum close to serotonergic pathways and recorded EEG and EMG continuously for 2 to 4 post-operative days in order to ascertain the time the animals spent awake and in different stages of sleep. They also analyzed brain serotonin and found a significant positive correlation between cortical serotonin and the time spent in SWS 2 and in REM, and a significant negative correlation between cortical serotonin stores and time spent in SWS 1. It thus appears that functional consequences ensue the significant decrease in brain serotonin in the pyridoxine-deficient rat.

NEUROTRANSMITTER RECEPTORS

Experimental conditions that increase intrasynaptic neurotransmitter concentration decrease post-synaptic receptor sensitivity and conversely experimental conditions that decrease neurotransmitter concentration lead to enhanced post-synaptic sensitivity (29). We have examined the effects of pyridoxine deficiency on dopamine, its high-affinity D-1 and D-2 receptors in the corpus striatum and for serotonin, its high affinity S-1 and S-2 receptors in the

cerebral cortex of rats. In the cerebral cortex of pyridoxine-deficient rats, a decrease of 54% in PLP resulted in a 47% decrease in serotonin content. In the same animals, there was a 71% decrease in the PLP content of the corpus striatum with no significant difference in its dopamine content when compared to pyridoxine-supplemented rats. Scatchard analysis of [^{3}H]-5-hydroxytryptamine binding to membrane preparations from cerebral cortex of deficient and supplemented rats indicates a significant increase in serotonin S-1 receptor concentration with a significantly decreased binding affinity (Table 6). Similar analysis of [^{3}H]-ketanserin binding to cerebral cortex membrane preparations shows an increase in B_{max} with a lowered binding affinity for the S-2 receptors (Table 6). 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 physiological function seem to be impaired in these animals in view of the low synaptic concentration of the neurotransmitter. Membrane preparations from corpus striatum of deficient and control rat brains were used to study the bindings, respectively, of [^{3}H]fluphenazine

Table 6. [^{3}H]-5-Hydroxytryptamine and [^{3}H]-Ketanserin Binding in the Cerebral Cortex of 3-Week Old Rat

Experimental Group	Control	Pyridoxine-deficient
[^{3}H]-5-Hydroxytryptamine binding[a]		
B_{max}, fmol/mg protein	934 ± 44	1799 ± 129[b]
K_d, nM	10.02 ± 0.78	20.12 ± 2.06[c]
[^{3}H]-Ketanserin binding[a]		
B_{max}, fmol/mg protein	217 ± 18	306 ± 21[d]
K_d, nM	0.69 ± 0.07	1.09 ± 0.11[d]

[a]Means ± S.E.M. were determined from 8 separate experiments each assayed in triplicate.
[b]$P<0.001$, [c]$P<0.01$, [d]$P<0.025$ with respect to control.

and [^{3}H]spiroperidol. Scatchard analysis of the binding data showed that there was no difference between pyridoxine-deficient and pyridoxine-supplemented rats either in the receptor concentrations or in the receptor binding affinities (Table 7). In both instances, receptor sensitivity seems to correlate negatively with the corresponding neurotransmitter concentrations in the pyridoxine-deficient rats (30).

Table 7. [^{3}H]-Spiroperidol and [^{3}H]-Fluphenazine Binding in the Corpus Striatum of 3-Week Old Rat

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

[a]Means ± S.E.M. were determined from 8 separate experiments each assayed in triplicate.

The high affinity binding of [^{3}H]-γ-aminobutyric acid to $GABA_A$ receptors and [^{3}H]baclofen to $GABA_B$ receptors were studied in membrane preparations from the cerebellum of pyridoxine-deficient rats and compared to pyridoxine-supplemented controls. Cerebellum was chosen for GABA binding studies as this region contains at least four cell types which utilize GABA as an inhibitory transmitter (31). In this brain region a 46% decrease in PLP resulted in a 56% decrease in its GABA content. There was a significant increase in the B_{max} of both $GABA_A$ and $GABA_B$ receptors with no significant difference in their binding affinities (Table

8). The supersensitivity of the $GABA_A$ and $GABA_B$ receptors seems to correlate negatively with the concentration of GABA in the cerebellum. GABAergic neurotransmission is impaired in spite of receptor supersensitivity because of the low synaptic concentration of GABA.

Table 8. [^{3}H] γ-Aminobutyric Acid (GABA) and [^{3}H]-Baclofen Binding in the Cerebellum of 3-Week Old Rat

Experimental Group	Control	Pyridoxine-deficient
[^{3}H]GABA binding[a]		
B_{max}, fmol/mg protein	961 ± 58	1728 ± 62[b]
K_d, nM	10.01 ± 0.63	10.82 ± 0.83
[^{3}H]Baclofen binding[a]		
B_{max}, fmol/mg protein	2.18 ± 0.13	3.52 ± 0.20[b]
K_d, nM	66.67 ± 7.74	73.73 ± 9.25

[a]Means ± S.E.M. were determined from 8 separate experiments each assayed in triplicate.
[b]$P<0.001$ with respect to control.

HYPOTHALAMIC-PITUITARY-THYROID AXIS

The nature of the defect in myelination in the pyridoxine-deficient rat closely parallels that seen in the hypothyroid animal. The central regulation of thyroid hormone secretion by monoamine neurotransmitters through the hypothalamic-pituitary pathway has been documented by many investigators (32-35). It is generally accepted that dopamine and serotonin have antagonistic effects (36). A direct relationship between serotonin turnover and thyroid-stimulating hormone (TSH) release has been established by Smythe et al. (37). In the pyridoxine-deficient animal we have an animal model in which various areas of the brain have de-

creased serotonin content with no change in the catecholamines. Both serotonergic and GABAergic neurotransmissions are compromised. In view of this, we investigated the neurotransmitter concentrations in the hypothalamus of the deficient rat. The results, given in Table 9 indicate that a

Table 9. Pyridoxal 5'-phosphate, Serotonin, Dopamine and Noradrenaline Contents in Control and Pyridoxine-Deficient Rat Hypothalamus

Animal Status	Supplemented (Control)	Pyridoxine-Deficient
Pyridoxal 5'-phosphate, nmol/g	2.71 ± 0.19	1.17 ± 0.07[a]
Serotonin, nmol/g	1.70 ± 0.20	1.00 ± 0.27[b]
Dopamine, nmol/g	1.18 ± 0.07	1.25 ± 0.10
Noradrenaline, nmol/g	2.17 ± 0.09	2.01 ± 0.10

Mean ± S.E.M. of 8 separate determinations in each group. [a]$P<0.001$, [b]$P<0.01$ compared with controls. (Student's unpaired t-test).

57% decrease in PLP in rat hypothalamus results in a 40% reduction in the hypothalamic serotonin with no significant change in the contents of dopamine and norepinephrine. The thyroid status of pyridoxine-deficient rats was compared with those of pair-weighed pyridoxine-supplemented controls and ad libitum fed normal controls. The concentrations of serum thyroid hormones (T_3 and T_4) of deficient rat pups were significantly lower in comparison with both pyridoxine-supplemented controls. There was no significant change from normal in the concentration of TSH in the serum of the deficient rats. However, pups in the deficient group had a significantly lower concentraton of pituitary TSH expressed per mg protein or per pituitary (Table 10). Daily intraperitoneal injection of thyrotropin-releasing hormone for one week to the deficient rats resulted in an increase in pituitary TSH concentration comparable to that seen in normal con-

trols. The fine structure of thyroid follicular cells from pyridoxine-deficient rats was similar to those seen in the control animals with the exception that the rough-surfaced endoplasmic reticulum was far less frequently observed in the markedly dilated form indicating perhaps a decrease in the synthesis of thyroglobulin. Thyrotroph cells from the pituitary of deficient rats were similar in fine structural appearance to those from control rats. Morphometric analysis of the numbers of secretory granules however, indicated a significant decrease in secretory granules in the thyrotrophs of deficient rats. These results are interpreted to indicate a hypothyroid condition of hypothalamic origin in the deficient rat, contributed by the decreased serotonergic neurotransmission.

Table 10. Serum Thyroxine (T_4), Tri-Iodothyronine (T_3), Thyroid Stimulating Hormone (TSH) and Pituitary TSH in Normal, Control and Pyridoxine-Deficient 3-Week Old Rat

	Normal (Group 1)	Control (Group 2)	Pyridoxine Deficient (Group 3)
T_4 (nM)	87.52±3.76 (19)	82.98±1.88 (40)	58.39±2.66[a] (41)
T_3 (nM)	1.54±0.07 (20)	1.40±0.06 (42)	0.98±0.03[a] (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[b] (41)	4.68±0.32[a] (38)
Pituitary TSH (μg/pituitary)	2.12±0.15 (17)	1.80±0.12 (41)	1.05±0.05[a] (38)

[a] $P<0.01$ compared with group 1 and 2, respectively.
[b] $P<0.05$ compared with group 1 (Duncan's multiple range test).
(N) number of experiments. Values represent mean ± S.E.M.

BLOOD PRESSURE REGULATION

Catecholamines acting directly on the periphery have a pressor effect. However, some α-adrenergic pathways in the CNS reduce the sympathetic tone and decrease blood pressure (38). The interaction between serotonergic and noradrenergic neurotransmissions has been suggested in various studies. Studies with 5-hydroxytryptophan and serotonin also indicate a depressor response for central serotonergic transmission (39). It is not clear whether serotonergic transmission alters blood pressure by modulating hypothalamic adrenergic blood pressure mechanisms or has a direct effect on brain stem autonomic centers. Various studies strongly suggest that central GABAergic transmission has hypotensive effects (40-42). Endocrine abnormalities have been linked with development of hypertension in spontaneously hypertensive rats. The most convincing correlation between hypothyroidism and hypertension has come from thyroid replacement studies in humans (43,44). In view of the pronounced hypothyroidism in pyridoxine-deficient rats, we examined arterial blood pressure in 12 week-old pyridoxine-deficient rats and compared them with ad libitum fed as well as pair-weighed pyridoxine-supplemented rats. There was a considerable degree of hypertension in the deficient rats. This was reversed by treatment of deficient rats with pyridoxine (Table 11). The hypertension of deficient rats was not related to the generalized malnutrition and more likely was related to the hypothyroid state of these animals.

The clinical effects of pyridoxine deficiency as they pertain to nervous system function can be explained on the basis of decreases in the activities of three enzymes (glutamic acid decarboxylase, 5-hydroxytryptophan decarboxylase and ornithine decarboxylase). The effects of the deficiency are quite devastating in the growing animal during the critical period in the development of the nervous system. Deficiency during the last week of gestation in the rat results in pups that cannot thrive for more than two or three weeks. Even a moderate degree of maternal pyridoxine deficiency during the lactation period affects the neurological development of the pups drastically. Maturation of the nervous system is impaired. Decreases in polyamines resulting from the decreased ornithine decarboxylase might be responsible for the impairment in protein synthesis. In addition, the decreases in specific neurotransmitters - GABA and serotonin - impair the normal neuroendocrine regulation. The hypothy-

roid condition of the young animal affects growth severely. Much of our evidence was obtained from studies on rat pups where deficiency was induced during the lactation period. During this period the syntheses of neurotransmitters as well as the formation of neuronal structures are affected. Even when pyridoxine deficiency is induced in the adult rat, the effects on the syntheses of neurotransmitters and their consequences are quite significant. The hypothyroid status of these animals and the consequent hypertension indicates the effect of compromised serotonergic and GABAergic neurotransmission on the hypothalamic-pituitary- thyroid axis.

Table 11. Systolic Blood Pressure in Experimental Groups

Group No.	Animal Status	Weight (g)	Systolic Blood Pressure (mm Hg)
1	Pyridoxine-supplemented (ad libitum fed; n=10)	472 ± 25	143 ± 7
2	Pyridoxine-supplemented (pair weighed; n=6)	146 ± 9	110 ± 3
3	Pyridoxine-deficient (ad libitum fed; n=15)	149 ± 15	185 ± 13
4	Pyridoxine-deficient rats fed pyridoxine-supplemented diet for 3 days (n=6)	172 ± 20	157 ± 11
5	Pyridoxine-deficient rats fed pyridoxine-supplemented diet for 6 days following 10 mg pyridoxine i.p. (n=6)	220 ± 31	149 ± 8

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