Sympathetic Stimulation and Hypertension in the Pyridoxine-Deficient Adult Rat

CHERAMADATHIKUDYIL S. PAULOSE, KRISHNAMURTI DAKSHINAMURTI,
SUBAH PACKER, AND NEWMAN L. STEPHENS

SUMMARY Pyridoxal phosphate is the coenzyme of various decarboxylases involved in the formation of monoamine neurotransmitters such as γ-aminobutyric acid, serotonin, dopamine, and norepinephrine. Adult male Sprague-Dawley rats placed on a pyridoxine-deficient diet for 8 weeks showed significant hypertension compared with pyridoxine-supplemented controls. Hypothalamic contents of pyridoxal phosphate, γ-aminobutyric acid, and serotonin in the pyridoxine-deficient rats were significantly lower than those in pyridoxine-supplemented controls. Hypertension was associated with sympathetic stimulation. Treatment of pyridoxine-deficient rats with a single dose of pyridoxine (10 mg/kg body weight) reversed the blood pressure to normal levels within 24 hours, with concomitant restorations of hypothalamic serotonin and γ-aminobutyric acid as well as the return of plasma norepinephrine and epinephrine to normal levels. Also, pyridoxine treatment reversed the hypothalamic hypothyroidism observed in pyridoxine-deficient rats. These results indicate an association between pyridoxine deficiency and sympathetic stimulation leading to hypertension.
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THE crucial role played by pyridoxine in the nervous system is evident from the fact that the putative monoamine neurotransmitters such as γ-aminobutyric acid (GABA), serotonin (5-hydroxytryptamine; 5-HT), dopamine (DA), and norepinephrine (NE) are formed through decarboxylation of the precursor amino acid or amino acid derivative. We have reported a decrease in the brain levels of GABA and 5-HT in the pyridoxine-deficient young rat, with no changes in the levels of the catecholamines.[1,2] Nonparallel changes in serotonin and dopamine are related to the heterogeneity of the decarboxylases for 5-hydroxytryptophan and dihydroxyphenylalanine.[3] Receptor, behavioral, and sleep studies reported by us attest to the functional consequences of the decrease in serotonin and GABA in various

areas of the rat brain.[4–6] We have also demonstrated the hypothalamic origin of hypothyroidism in the pyridoxine-deficient rat.[7] Various reports have indicated a relationship between pyridoxine status and hypertension in pregnant women and women taking anovulatory steroids.[8–10] Although an increase in systolic blood pressure has been reported in pyridoxine deficiency,[11] it has not been studied systematically. In the present report, we elucidate the sympathetic stimulation and hypertension occurring in pyridoxine deficiency.

Materials and Methods

Animals

Adult male Sprague-Dawley rats (age, 6 weeks; weight, 139 ± 8 g; Charles River, St.-Constant, PQ, Canada) were used in these experiments. They were divided randomly into three groups: Group 1 was fed laboratory chow ad libitum, Group 2 was fed a pyridoxine-supplemented (control) diet, and Group 3 was fed a pyridoxine-deficient diet ad libitum. The rats in Group 2 were pair-fed with pyridoxine-deficient rats.

Blood Pressure Measurements

Tail-Cuff Plethysmography

Systolic blood pressure was recorded weekly using tail-cuff plethysmography. At the end of the 8-week

experimental period, rats in each of the three groups were subdivided into two groups. One subgroup was injected with pyridoxine (10 mg/kg body weight i.p.), and the other with saline. Systolic blood pressures were recorded after 24 hours.

Direct Arterial Measurement

Pyridoxine-supplemented (control) and pyridoxine-deficient rats were used for cannulation. Rats were anesthetized with urethane (100 mg/100 g body weight i.p.). The trachea was intubated to maintain adequate ventilation. The right carotid artery was exposed and cannulated for recording of blood pressure. A 21-gauge 1-in. needle with a blunt tip was fixed to the pressure transducer, which was connected in turn to a Beckman Dynograph recorder R511A (Berkeley, CA, USA) to record the arterial pressure. The damping effect on the blood pressure due to a long and narrow catheter was corrected by directly attaching the needle to the pressure transducer.

Chronic Catheterization for Blood Sampling

Adult rats fed pyridoxine-supplemented and pyridoxine-deficient diets for 8 weeks were prepared for long-term catheterization using a vascular access port (Model SLA, Norfolk Medical Products, Skokie, IL, USA). Blood samples were collected with minimal stress to the animals. The blood samples were centrifuged at 5000 g for 10 minutes, and the plasma collected was stored at $-70°C$ until it was used for NE and epinephrine (E) determinations.

Determinations of Pyridoxal Phosphate and Neurotransmitters

Pyridoxal phosphate was determined using tyrosine apodecarboxylase as described previously.[12] 5-HT, DA, and NE in the brain were assayed radioenzymatically.[13,14] GABA was determined using the radioreceptor assay as described by Frere et al.[15] Plasma NE and E were determined using high performance liquid chromatography (HPLC) with electrochemical detection as developed in our laboratory.

Extraction of Catecholamines from Plasma

For the extraction of catecholamines from plasma, 1.0 ml of plasma was transferred to a tube containing 1.0 ml of distilled water. Then, 50 μl of 5 mM sodium bisulfite was added and mixed, followed by 250 μl of 1 M Tris buffer, pH 8.6. Acid alumina (20 mg) was then added. The contents of the tube were mixed using a rotator for 20 minutes. The supernatant was aspirated using a Pasteur pipette. The alumina was washed twice with 2 ml of water containing 5 mM sodium bisulfite. To the final pellet of alumina, 0.2 ml of 0.1 N perchloric acid was added and mixed in a vertical mixer for 15 minutes. The supernatant then was used for HPLC determination of catecholamines.

HPLC Separation Conditions

The extracted sample (20 μl) was injected into a Beckman HPLC apparatus with an Altex C18-1P re-verse-phase column (inside diameter, 25 cm × 4.6 mm; 5 μm particle size; Beckman). The mobile phase consisted of 75 mM sodium phosphate monobasic, 1 mM sodium octylsulfate, 50 μM EDTA, and 11.5% acetonitrile. The buffer was adjusted to pH 3.25 with phosphoric acid, filtered, and deaerated before use. A flow rate of 1 ml/min was used with a Beckman Model 114 solvent delivery module. The catecholamines were identified by coulometric detection using an ESA Model 5100 A detector (Bedford, MA, USA) with Detector 1 set at a reduction potential of 0.05 V and Detector 2 set at an oxidation potential of 0.40 V. A preinjector guard cell was set at 0.45 V. The peaks were identified by relative retention times compared with standards, and concentrations were determined by comparing peak areas using a Shimadzu integrator (Columbia, MD, USA) interfaced with the detector.

Assay of Hormones

Pituitary and serum thyroid stimulating hormone (TSH) were assayed using reagents and protocol provided by NIADDK (Bethesda, MD, USA). The TSH values were expressed in terms of the RP-2 standard, which is 176 times more potent than the NIADDK-rTSH-RP-1 standard previously supplied. Concentrations of thyroxine (T_4) and triiodothyronine (T_3) were determined using T_4 and T_3 solid-phase radioimmunoassay kits purchased from Becton-Dickinson (Orangeburg, NY, USA). Serum T_4 and T_3 concentrations were expressed in nanomoles per liter. Protein was measured according to the method of Lowry et al.[16]

Statistical Analysis

The data from different groups of animals were analyzed statistically by analysis of variance followed by Duncan's multiple range test.

Results

The mean body weight (in grams) versus time (weeks on the respective diet) curves for the pyridoxine-deficient and control rats are presented in Panel B of Figure 1. Panel A shows the mean systolic blood pressure (mm Hg) versus time (weeks on the respective diet) curves for these rats during the experimental period. The blood pressure of pyridoxine-deficient rats had increased ($p < 0.005$) by the 5th week, and by the end of the experimental period, the values ($143 ± 6$ mm Hg) were still significantly ($p < 0.005$) higher compared with control values ($123 ± 3$ mm Hg). Twenty-four hours after pyridoxine treatment, the blood pressure of the pyridoxine-deficient rats dropped significantly to $119 ± 4$ mm Hg. There was no significant difference between the blood pressure values of the control and pyridoxine-injected control rats.

Direct measurement of arterial pressure by carotid artery catheterization indicated a significant increase in both systolic and diastolic blood pressures of the pyridoxine-deficient rats when compared with those of the controls (Table 1).

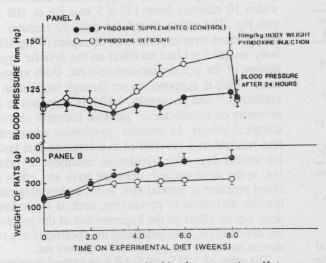Hypothalamic contents of pyridoxal phosphate,

FIGURE 1.   *A. Mean systolic blood pressure (mm Hg) versus time (weeks) for pyridoxine-deficient (n = 20) and control (n = 15) rats.   B. Mean body weight (g) versus time (weeks) for the two groups. SE bars are also given. The test rats were maintained on a pyridoxine-deficient diet from Weeks 0 to 8, while the control rats were maintained on a pyridoxine-supplemented diet throughout this period. At the end of the 8th week, control and pyridoxine-deficient rats were injected with pyridoxine, 10 mg/kg, and blood pressures were measured 24 hours later.*

TABLE 1.   *Arterial Blood Pressure of Pyridoxine-Deficient and Pyridoxine-Supplemented (Control) Rats*

Animal status	Diastolic arterial pressure (mm Hg)	Systolic arterial pressure (mm Hg)
Pyridoxine-supplemented (control)	77 ± 3	111 ± 2
Pyridoxine-deficient	$105 \pm 6^*$	$147 \pm 5\dagger$

Values are means $\pm$ SEM of five separate determinations in each group.

$^*p < 0.005$, $\dagger p < 0.001$, compared with control (by Student's unpaired t test).

GABA, and 5-HT of pyridoxine-deficient rats were significantly ($p < 0.01$) lower than those of control rats (Table 2). DA and NE contents of the hypothalamus were not decreased in the pyridoxine-deficient rats. There was minimal stress to the rats when blood was drawn through the vascular access port. This is indicated by a comparison of plasma catecholamine of blood collected from the vascular access port with that obtained by decapitation of the animal. NE and E levels in the peripheral plasma of pyridoxine-deficient rats were significantly ($p < 0.01$) higher compared with those of the controls (Table 3). Treatment of hypertensive rats with pyridoxine restored hypothalamic pyridoxal phosphate, GABA, 5-HT, and serum NE and E within 24 hours to normal levels (see Tables 2 and 3). Pyridoxine treatment of the control (pyridoxine-supplemented) rats had no significant effect on any of these parameters.

Pyridoxine-deficient rats showed a significant decrease in serum TSH, T_4 ($p < 0.01$), and serum T_3 ($p < 0.05$). There was also a significant increase in pituitary TSH content ($p < 0.01$) in the pyridoxine-deficient rats (Table 4). Pyridoxine injection to pyridoxine-deficient rats reversed the hypothyroidism observed due to pyridoxine deficiency within 24 hours.

Discussion

Our results indicate that pyridoxine deficiency in the adult male rat leads to true arterial hypertension, which is reversed within 24 hours by pyridoxine treatment. This complete reversal within such a short time would exclude vessel wall damage as the primary cause of the hypertension.

Treatment of pyridoxine-deficient rats with pyridoxine restored systolic blood pressure, serum TSH, T_4, T_3, NE, E, pituitary TSH, and hypothalamic 5-HT and GABA to normal levels within 24 hours. The plasma renin activity of the pyridoxine-deficient rats was still elevated 24 hours after pyridoxine treatment (control, 1.03 ± 0.24 pmol/L/sec; pyridoxine-deficient, 2.38 ± 0.20 pmol/L/sec; pyridoxine-injected pyridoxine-deficient, 2.23 ± 0.33 pmol/L/sec), excluding a primary renal cause of hypertension in these animals.

Pyridoxine deficiency is characterized by slow

TABLE 2.   *Effect of Pyridoxine on Pyridoxal Phosphate, γ-Aminobutyric Acid, Serotonin, Dopamine, and Norepinephrine Contents in the Hypothalamus of Control and Pyridoxine-Deficient Adult Rats*

Animal status	Pyridoxal phosphate (nmol/g)	GABA (µmol/g)	Serotonin (nmol/g)	Dopamine (nmol/g)	Norepinephrine (nmol/g)
Group 1: pyridoxine-supplemented (control)	3.49 ± 0.26	5.33 ± 0.16	1.38 ± 0.05	1.06 ± 0.20	3.34 ± 0.20
Group 2: pyridoxine-treated (control)	3.26 ± 0.19	5.46 ± 0.21	1.49 ± 0.06	1.12 ± 0.16	3.29 ± 0.14
Group 3: pyridoxine-deficient (experimental)	$1.89 \pm 0.16^*$	$4.34 \pm 0.16^*$	$0.92 \pm 0.04^*$	1.13 ± 0.18	3.03 ± 0.40
Group 4: pyridoxine-treated (experimental)	3.06 ± 0.16	5.32 ± 0.21	$1.85 \pm 0.13\dagger$	1.21 ± 0.22	3.25 ± 0.28

Values are means $\pm$ SEM of 10 separate determinations in each group. GABA = γ-aminobutyric acid.

$^*p < 0.01$, compared with Groups 1, 2, and 4; $\dagger p < 0.01$, compared with Groups 1, 2, and 3 (by Duncan's multiple range test).

TABLE 3. *Effect of Pyridoxine on Plasma Levels of Norepineph-rine and Epinephrine in Control and Pyridoxine-Deficient Adult Rats*

Animal status	Norepinephrine (nmol/L)	Epinephrine (nmol/L)
Group 1: pyridoxine-supple-mented (control)	3.06 ± 0.28	1.89 ± 0.28
Group 2: pyridoxine-treated (control)	3.44 ± 0.27	1.52 ± 0.16
Group 3: pyridoxine-deficient (experimental)	$9.04 \pm 0.21*$	$4.39 \pm 0.21*$
Group 4: pyridoxine-treated (experimental)	3.97 ± 0.32	2.73 ± 0.24

Values are means $\pm$ SEM of eight to 12 separate determinations in each group.

$*p < 0.01$, compared with Groups 1, 2, and 4 (by Duncan's multiple range test).

growth and suppressed appetite in rats. Hence, it could be questioned whether the observed hypertension in pyridoxine-deficient rats is a consequence of malnutri-tion of the deficient rats. To answer this question, in another experiment we compared control rats (pyri-doxine-supplemented rats that were pair-fed with pyridoxine-deficient rats) with pyridoxine-deficient rats to examine the effect of generalized malnutrition on blood pressure. Rats subjected to generalized mal-nutrition had significantly lower blood pressure (87 ± 4 mm Hg) compared with controls (114 ± 5 mm Hg) and pyridoxine-deficient rats (149 ± 10 mm Hg). Thus, the hypertension seen in pyridoxine-deficient rats was not a consequence of generalized malnutrition in these rats.

Pyridoxine deficiency in the rat could also result in a hyperexcitable state. Seizures are seen in the pyridox-ine-dependent state as well as in experimental pyridox-ine deficiency in young rats. Although spontaneous seizures were not observed in adult pyridoxine-defi-cient rats, we investigated the effects of anticonvulsant drugs such as phenytoin, valproic acid, and diazepam on the blood pressure of these rats. A single dose of phenytoin (6 mg/100 g body weight i.p.) decreased the systolic blood pressure in pyridoxine-deficient rats within 30 minutes from 135 ± 4 mm Hg to 105 ± 3 mm Hg. The effect lasted for 6 hours, at the end of which the blood pressure was elevated again. Valproic

acid (16 mg/100 g body weight i.p.) reversed the high systolic blood pressure in the pyridoxine-deficient rats within 10 minutes from 133 ± 3 mm Hg to 108 ± 2 mm Hg. The effect lasted for only 30 minutes. In similar short-term experiments, diazepam (8 mg/100 g body weight i.p.) had no effect on the systolic hyper-tension of the pyridoxine-deficient rat. Both valproic acid, which is supposed to act through facilitation of inhibition,[17] and phenytoin, which is supposed to act primarily on membranes,[18] produce transient pharma-cological effects. In contrast, pyridoxine administra-tion resulted in a reversal of hypertension that lasted for several days after pyridoxine treatment. Pyridox-ine, at the dose studied, does not have any effect on blood pressure in normal rats. Also, a physiologically inactive derivative of pyridoxine, such as 4-pyridoxic acid, has no effect on the hypertension of the pyridox-ine-deficient rat, thus indicating the specificity of pyri-doxine action in the pyridoxine-deficient rat.

The roles of 5-HT and GABA in central regulation of blood pressure have been studied.[19, 20] Hypothyroid-ism is also known to cause hypertension, although the mechanism of this action is not known.[21] We also in-vestigated the possibility that the reversible hyperten-sion seen in the pyridoxine-deficient rats is related to general sympathetic stimulation. The concentration of NE in peripheral plasma can be taken as reflecting sympathetic activity.[22] The significant increase in se-rum NE in the pyridoxine-deficient rats indicates gen-eral sympathetic stimulation. The sympathetic hyper-activity could be due to the effect of pyridoxine deficiency at the sympathetic nerve terminals and in the adrenal gland itself.

Dahlstrom and Fuxe[23] have demonstrated the coinci-dence of medullary indoleamine tracts with neural pathways controlling cardiovascular function. In con-sidering the central serotoninergic influence on blood pressure, the peripheral effects cannot be ignored. The current controversy about the role of central serotonin-ergic neurons in the control of blood pressure can be attributed to the tendency to treat them as a homoge-neous network subserving a single function. Chalmers et al.[24] stress that the medial elements of the B_1 and B_5 serotoninergic cell groups contained within the medul-lary raphe nuclei may have a depressor action. It has been suggested that the hypotensive effect of long-

TABLE 4. *Effect of Pyridoxine on Pituitary and Serum Thyroid Stimulating Hormone and Serum Thyroxine and Triiodothyronine in Control and Pyridoxine-Deficient Adult Rats*

Animal status	Pituitary TSH (μg/mg protein)	Serum TSH (μg/L)	Serum T_4 (nmol/L)	Serum T_3 (nmol/L)
Group 1: pyridoxine-supplemented (control)	2.39 ± 0.18	3.91 ± 0.28	89 ± 5	0.98 ± 0.04
Group 2: pyridoxine-treated (control)	2.43 ± 0.19	4.19 ± 0.82	92 ± 8	1.00 ± 0.06
Group 3: pyridoxine-deficient (experimental)	$5.90 \pm 0.48*$	$1.94 \pm 0.61*$	$64 \pm 9†$	$0.84 \pm 0.08†$
Group 4: pyridoxine-treated (experimental)	3.70 ± 0.27	3.46 ± 0.63	90 ± 3	1.15 ± 0.07

Values are means $\pm$ SEM of eight to 12 separate determinations in each group. TSH = thyroid stimulating hormone; T_4 = thyroxine; T_3 = triiodothyronine.

$*p < 0.01$, $†p < 0.05$, compared with Groups 1, 2, and 4 (by Duncan's multiple range test).

term 5-hydroxytryptophan infusion is due to an action in the brainstem.[25] Serotonin may participate as a modulator of sympathetic activity. Various hypertensive states share as a common factor an increased sympathetic outflow. Intracerebroventricular injection of norepinephrine and other α-agonists has been shown to reduce blood pressure and heart rate in a number of species.[26] α-Adrenergic receptors in the hypothalamus and brainstem appear to mediate this effect.[27] An interaction between serotoninergic and noradrenergic neurotransmission has been suggested, taking into account the anatomical proximity of both pathways.

Central GABAergic transmission has been suggested to cause hypotensive effects. Intracerebroventricular injection of GABA produces a hypotensive response[28] that is blocked by bicuculline.[20] The bilateral microinjection of bicuculline into the ventrolateral vasopressor neuron pool causes a dose-related increase in blood pressure, pulse pressure, and heart rate.[29] Administration of GABA or its agonists such as muscimol to the brain causes a reduction in blood pressure and heart rate in several species.[30, 31] A reduced sympathetic outflow has been implicated in the mediation of the cardiovascular effects of GABA.[32] Hence, we speculate that the decreased serotoninergic and GABAergic central neurotransmission in the pyridoxine-deficient rat, acting through stimulation of sympathetic outflow, could cause the reversible hypertension in this animal model.

Acknowledgment

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