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## Alterations in brainstem $\alpha_2$ adrenoreceptor activity in pyridoxine-deficient rat model of hypertension

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Moderate pyridoxine deficiency in adult male Sprague-Dawley rats results in significant hypertension, associated with a general sympathetic stimulation, including an increase in the turnover of norepinephrine in the heart. Treatment of these rats with pyridoxine reversed blood pressure to normal within 24 h. Treatment of pyridoxine-deficient rats with clonidine or  $\alpha$ -methyl dihydroxyphenylalanine ( $\alpha$ -methyl DOPA) also reduced the blood pressure of these animals to normal. There was also a significant increase in the  $B_{max}$  of high and low affinity [ $^3$ H] $p$ -amino-clonidine binding to crude synaptosomal membrane preparations of the brain stem of deficient rats indicating chronic underexposure of  $\alpha_2$  adrenoreceptors to endogenous norepinephrin.

We have reported [12] that moderate deficiency of pyridoxine in the adult male Sprague-Dawley rat resulted in a significant hypertension. Hypothalamic contents of pyridoxal phosphate,  $\gamma$ -aminobutyric acid (GABA) and serotonin of the deficient rats were significantly lower than those of pyridoxine-supplemented controls. Plasma norepinephrine and epinephrine levels were determined on blood samples withdrawn from conscious animals using a vascular access port [11]. These values were significantly increased in the pyridoxine deficient rats as compared to pyridoxine supplemented controls. Treatment of the pyridoxine-deficient rats with a single dose of pyridoxine restored systolic blood pressure to normal. Concomitant with this, hypothalamic serotonin and GABA as well as plasma catecholamines were returned to normal levels.

The roles of serotonin and GABA in central regulation of blood pressure have been discussed [6, 13]. The incidence of medullary serotonin tracts with normal pathways controlling cardiovascular function has been shown by Dahlstrom and Fuxe [3]. The hypotensive effect of long term 5-hydroxytryptophan infusion has been suggested to be due to an action at the brainstem [7]. Serotonin may participate as a

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modulator of sympathetic activity. Increased sympathetic outflow is a common feature of various hypertensive conditions. In this report we have examined the possibility that pyridoxine deficiency-induced increase in peripheral sympathetic activity results from altered noradrenergic transmission in the brainstem.

Adult male Sprague-Dawley rats (100–120 g) were maintained on a pyridoxine-deficient diet [4] for 8 weeks at which time moderate deficiency of pyridoxine was noticed in these animals. The control group of rats were pair-weighted on a pyridoxine-supplemented diet for the same duration. In initial experiment norepinephrine (NE) turnover in the heart in both deficient and control groups were determined by estimating the decrease in NE content in the heart after inhibition of its synthesis with alpha-methyl p-tyrosine [1, 17]. NE was determined using the HPLC technique [12]. The results given in Table I indicate that there was no difference in the myocardial NE content between the deficient and control groups. However, NE turnover in the heart was increased significantly ( $P < 0.05$ ) in pyridoxine-deficient animals when compared to controls supporting the earlier observations that peripheral sympathetic activity is increased in these animals. The effect of anti-hypertensive drugs – clonidine and  $\alpha$ -methyl dihydroxyphenylalanine(-methyl DOPA) were tested in pyridoxine-deficient rats fitted with vascular access port (Model SLA, Norfolk Medical Products, Skokie, IL, U.S.A.). Basal systolic blood pressure of the deficient rats recorded using tail cuff plethysmography was stable. Following this, drugs were administered through the port twice daily at 09.00 and 17.00 h for 4 days. Clonidine hydrochloride (10  $\mu$ g/kg b.wt.) and  $\alpha$ -methyl DOPA (40 mg/kg b.wt.) were given to separate groups of deficient rats. The third group of deficient rats continued to receive the vehicle (normal saline) through the port for the equivalent time period. Blood pressure recordings were taken on day 5. Clonidine and  $\alpha$ -methyl DOPA both reduced the blood pressure of deficient animals to normal (Table II). Clonidine like drugs exert their cardiovascular depressive effect mainly through a centrally mediated sympatho-inhibition due to a stimulation of  $\alpha$ -adrenoreceptors [8].

TABLE I

MYOCARDIAL NOREPINEPHRINE (NE) CONTENT AND TURNOVER RATES IN PYRIDOXINE-SUPPLEMENTED AND PYRIDOXINE-DEFICIENT ADULT RAT

Alpha-methyltyrosine (200 mg/kg) was injected into the tail vein of rats. At various time intervals thereafter rats were killed by cervical dislocation, hearts removed, rinsed free of blood and extracted with ice cold 0.4 N perchloric acid. NE was assayed using HPLC with electrochemical detection. Slope of decline was calculated by the method of least squares. Values represent mean  $\pm$  S.E.M. of 8 separate experiments.

	NE content (ng/g)	NE turnover rate (ng/g/h)
Pyridoxine-supplemented	1661.8 $\pm$ 241.8	30 $\pm$ 4.4
Pyridoxine-deficient	1955.0 $\pm$ 260.8	106.6 $\pm$ 14.2*

\* $P < 0.05$ .



TABLE II

EFFECT OF CLONIDINE AND  $\alpha$ -METHYL DOPA ON SYSTOLIC BLOOD PRESSURE OF PYRIDOXINE-DEFICIENT ADULT RATS

Values are mean  $\pm$  S.E.M. of 5 separate determinations.

	Blood pressure
Pyridoxine-deficient	134 $\pm$ 4*
Pyridoxine-deficient + clonidine (10 $\mu$ g/kg)	107 $\pm$ 3
Pyridoxine-deficient + $\alpha$ -methyl DOPA (40 $\mu$ g/kg)	105 $\pm$ 4

\* $P < 0.01$  compared with groups 2 and 3.

In view of the hypotensive effect of the  $\alpha_2$  receptor agonists the possibility of decreased adrenergic input to the brainstem was examined. The kinetics of ligand binding to  $\alpha_2$  adrenoreceptors were investigated using [ $^3$ H]*p*-aminoclonidine (PAC) binding to crude synaptosomal membrane preparations from the brainstem of pyridoxine-deficient and control rats according to Sripanidkulchai and Wyss [16] with modifications. Membranes were prepared by polytron homogenization (setting at 6) for 30 s in ice cold 50 mM Tris-HCl (pH 7.4). After centrifugation at 48,000 g for 10 min, the resulting pellet was resuspended in the ice-cold buffer and used for binding studies. The standard assay consisted of 0.2–0.4 mg protein of the brainstem membrane preparation, 0.1–25 nCi [ $^3$ H]PAC and the buffer with or without excess of unlabelled PAC (100  $\mu$ M) in a final volume of 0.5 ml. The mixture was incubated for 45 min at 25°C. The assay mixtures were filtered over glass fiber filters which were previously soaked in 0.3% polyethylenimine for 3 h and rapidly washed with 3  $\times$  5 ml ice-cold Tris-HCl buffer. [ $^3$ H]PAC bound to the membranes in the filter was determined by liquid scintillation spectrometry. The specific binding was determined by subtracting non-specific binding from the total binding. Specific binding data were analyzed according to Scatchard [15] from which maximal binding ( $B_{\max}$ ) and the dissociation constant ( $K_d$ ) were derived by linear regression analysis [10]. The results given in Table III show a significant increase in the  $B_{\max}$  of the high- and low-affinity

TABLE III

*p*[3,5- $^3$ H]AMINOCLOMIDINE BINDING TO THE ALPHA<sub>2</sub> - ADRENORECEPTORS IN THE BRAINSTEM OF CONTROL AND PYRIDOXINE-DEFICIENT ADULT RATS

Values are mean  $\pm$  S.E.M. of separate experiments.

Group	High-affinity	$K_d$	Low-affinity	$K_d$
	$B_{\max}$ (fmol/mg protein)	(nM)	$B_{\max}$ (fmol/mg protein)	(nM)
Control	51 $\pm$ 3	1.65 $\pm$ 0.33	172 $\pm$ 16	7.48 $\pm$ 1.41
Pyridoxine-deficient	89 $\pm$ 9*	1.96 $\pm$ 0.54	247 $\pm$ 9*	9.17 $\pm$ 0.47

\* $P < 0.01$  compared with the control group.

[<sup>3</sup>H]PAC binding to  $\alpha_2$  adrenoreceptors in the brainstem of pyridoxine-deficient rats compared with controls without any change in the binding affinity. This would indicate chronic underexposure of  $\alpha_2$  adrenoreceptors to endogenous NE. In support of such a possibility is the significant ( $P < 0.05$ ) decrease in the 3-methoxy-4-hydroxyphenylglycol (MHPG) levels in the brainstem of deficient rats ( $12.1 \pm 1.1$  ng/g in deficient vs  $19.4 \pm 1.7$  in controls), again indicating a lower turnover of NE in the brainstem.

The central mechanism of maintaining normal blood pressure in animals is regulated by the balance between sympathetic and parasympathetic nervous system tonicities in the brain stem [5, 9]. The anti-hypertensive effect of clonidine results from its pharmacological reactivity for central  $\alpha_2$  adrenoreceptors in the nucleus tractus solitarii (NTS). When  $\alpha_2$  adrenoreceptors in the nucleus tractus solitarii (NTS) are stimulated, inhibitory neurons of the vasomotor center are activated. Sympathetic outflow which originates from the vasomotor center and innervates the peripheral vasculature, heart and kidney is reduced. As a result, peripheral vascular tone, heart rate and renin release are decreased resulting in a decrease in total peripheral resistance and cardiovascular output [6]. Recent studies suggest that the regulation of central adrenergic receptors is not confined to adrenergic mechanisms alone but requires a serotonergic component [14]. As seen in the down regulation of  $\beta$  adrenergic receptors induced by anti-depressant drugs [2] modification of  $\alpha_2$  adrenergic receptor could require a degree of serotonergic input. Lesioning of the central serotonergic pathways using 5,7-dihydroxytryptamine led to an increase in the  $B_{\max}$  of [<sup>3</sup>H]PAC binding. In the pyridoxine-deficient rats there was a significant reduction in the serotonin content of the brainstem ( $3.88 \pm 0.14$  nM/g for deficient vs  $5.39 \pm 0.17$  for controls). The significant increase in the  $B_{\max}$  of Ketanserin binding to the serotonin  $S_2$  receptors on the crude synaptosomal membrane preparations of brainstem of the pyridoxine-deficient rat ( $157 \pm 8$  fmoles/mg protein in deficient vs  $122 \pm 11$  in controls) with no change in the binding affinity would further suggest a chronic underexposure of the brainstem to serotonergic input. It is probable that the decrease in serotonergic activity of the brainstem of pyridoxine-deficient rats is responsible for the alteration in  $\alpha_2$  adrenergic function and the resultant sympathetic stimulation.

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