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Influence of B Vitamins on Binding Properties of Serotonin Receptors in the CNS of Rats

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Summary. Treatment of normal adult rats with pyridoxine or a B-vitamin mixture resembling Neurobion®[1] led to an increase in serotonin content of various brain areas and to a decrease in the number of serotonin S_2 receptors. The results indicate that the pyridoxal phosphate level in regions of the brain regulates the extent of decarboxylation of 5-hydroxytryptophan, the precursor of serotonin. The results also suggest a continuum from deficiency in pyridoxine to treatment of animals with a moderate excess of pyridoxine which is reflected in the synthesis and secretion into the synaptic cleft of the neurotransmitter serotonin.

Key words: Pyridoxine – B Vitamins – CNS – Serotonin receptor binding – Rat

Vitamins in the B group have been used clinically along with antiinflammatory drugs in painful conditions such as neuralgia or inflammation. Recent animal work indicates that B-vitamin mixture dose-dependently potentiates the antinociceptive effect of diclofenac [1, 8]. The effect of a challenge of the brain by either clonidine or morphine was amplified after subacute treatment of rats with B vitamins for 1 week. The mode of action of the vitamin preparation (Neurobion) which includes thiamin, pyridoxine and cyanocobalamin is not clear. We have, however, studied the effect of pyridoxine alone which indicates that pyridoxine-deficient rats exhibited reduced nociceptive threshold as compared with pyridoxine-supplemented animals. Intraperitoneal administration of pyridoxine to both pyridoxine-deficient and control (pyridoxine-supplemented) rats resulted in significant reduction in the thalamic ventro posterior nucleus (VPL) nociceptive unit activity following electrical stimulation of nociceptive afferents in the ipsilateral sciatic nerve, although this effect was much less marked than that of morphine. A physiological function has been indicated for substance-P (SP) and serotonin (5HT) neurons of the dorsal raphe magnus in inhibition of spinal pain transmission involving postsynaptic receptors in the spinal cord innervated by the bulbospinal SP and serotonergic system [11]. The rationale for our investigation is that pyridoxine influences serotonin synthesis.

Material and Methods

Sprague Dawley rats (150 ± 5 g body weight) were given pyridoxine (100 mg/kg body weight) or a B-vitamin mixture resembling the composition of Neurobion (33.4 mg thiamin, 33.4 mg pyridoxine and 0.34 mg cyanocobalamin per kg body weight) in buffered saline intraperitoneally for 7 days. Control rats received similar i.p. injections of the vehicle only. The rats were killed 24 h after the last pyridoxine administration. Various regions of the brain were isolated according to Glowinski and Iversen [7]. Tissues were frozen immediately and stored at $-70°$ C till they were used. Serotonin was assayed using the HPLC method of Kilpartrick et al. [9]. Crude synaptic membranes from the various brain regions were isolated as described [12]. The kinetics of (3H)-ketanserin binding were assessed according to Leysen et al. [10]. The specific binding data were analysed according to Scatchard [13], using equalibrium binding data analysis (EBDA) software, from which the binding parameters, maximal binding (B_{max}) and dissociation constant (Kd) were determined.

[1] Neurobion®, Cascan GmbH & Co. KG, Wiesbaden, FRG

Results

The treatment of normal adult rats with moderate doses of pyridoxine or a B-vitamin mixture daily for 7 days led to an increase in the serotonin content in various areas of the brain. Both the serotonin content as well as the tissue response to pyridoxine treatment were heterogeneous (Table 1). It is to be noted that although the B-vitamin mixture contained only one-third the pyridoxine content of the "pyridoxine treatment", it elicited the same increase in the serotonin content in most brain regions, suggesting the possibility that this lower level of pyridoxine already gave an optimal increase in brain serotonin. An alternate possibility is a role for other components of the B-vitamin mixture in increasing brain serotonin. Serotonin S_2 receptor numbers of various brain areas were significantly lower in the animals treated with pyridoxine or the B-vitamin mixture (Table 2), indicating that the tissue serotonin content reflects the intrasynaptic concentration of the neurotransmitter. Thus, treatment of rats with moderate doses of pyridoxine results in an increment in brain serotonin indicating that the tissue 5HTP-decarboxylation responds to the pyridoxine status of the animal.

We have also examined the serotonin content and the serotonin S_2 receptor number in synaptosomal membrane preparations from the cerebral cortex of 3-week-old rats. Their pyridoxine status ranged from deficiency to moderate excess. There were four groups: (1) normal controls on ad libitum commercial pellet diet; (2) controls receiving pyridoxine (10 mg/kg body weight, i.p. daily for 7 days); (3) pyridoxine-deficient rats (dams were fed a pyridoxine-deficient diet during the lactation period); and (4) pyridoxine-deficient rats given

Table 1. Effect of pyridoxine or B-vitamin mixture treatment on 5-HT in different rat brain regions

Brain regions	Serotonin (nmole/g wet tissue)
Cerebral cortex	
Control	1.97±0.12
Pyridoxine	4.25±0.32*
B-Vitamin mixture	4.26±0.35*
Hippocampus	
Control	4.05±0.16
Pyridoxine	7.32±0.21*
B-Vitamin mixture	5.03±0.18*
Thalamus	
Control	3.21±0.14
Pyridoxine	5.15±0.29*
B-Vitamin mixture	4.14±0.22*
Hypothalamus	
Control	3.27±0.12
Pyridoxine	3.68±0.16*
B-Vitamin mixture	4.15±0.18*
Brain stem	
Control	3.00±0.15
Pyridoxine	4.41±0.17*
B-Vitamin mixture	4.16±0.15*
Cerebellum	
Control	0.84±0.15
Pyridoxine	1.80±0.18*
B-Vitamin mixture	1.81±0.23*
Spinal cord	
Control	1.13±0.07
Pyridoxine	1.33±0.08*
B-Vitamin mixture	1.33±0.07*

Data are mean±SEM, obtained from eight separate animals in each group

* $P<0.05$ (i) controls (saline injected), (ii) pyridoxine treated (100 mg/kg i.p. for 7 days), and (iii) B-vitamin mixture treated (33.4 mg thiamin, 33.4 mg pyridoxine and 0.34 mg cyanocobalamin per kg i.p. for 7 days)

Table 2. Effect of pyridoxine and B-vitamin mixture treatment on 5-HT_2 receptor binding in different rat brain regions

	B_{max} (f mole/mg protein)	Kd (n*M*)
Cerebral cortex		
Control	223±13.5	0.43±0.09
Pyridoxine	150±15.7*	0.46±0.07
B-Vitamin mixture	160±17.1*	0.49±0.05
Thalamus		
Control	221±17.5	0.73±0.03
Pyridoxine	175± 8.6	0.70±0.05
B-Vitamin mixture	196±12.1	0.74±0.06
Hypothalamus		
Control	220± 9.7	0.75±0.05
Pyridoxine	170± 7.5*	0.76±0.05
B-Vitamin mixture	188±10.5	0.78±0.07
Brain stem		
Control	157± 8.0	0.94±0.03
Pyridoxine	122±11.0*	0.93±0.06
B-Vitamin mixture	130± 5.0*	0.93±0.31
Spinal cord		
Control	230± 7.5	0.70±0.08
Pyridoxine	165± 8.5*	0.76±0.09
B-Vitamin mixture	180±11.6*	0.78±0.08

Data are mean±SEM, determined from eight separate experiments each assayed in triplicate

* $P<0.05$ with respect to control

B_{max} values were significantly reduced in pyridoxine or B-vitamin mixture treated animals. Kd for a particular brain tissue remained constant. For treatment see Table 1

Table 3. Correlation between 5-HT and the B_{max} of ^{3}H-ketanserin binding to 5-HT_2 receptors in the cerebral cortex

Experimental group	5-HT (nmole/g wet weight)	B_{max} of ^{3}H-ketanserin (fmole/mg) tissue protein)
Normal	1.12±0.13	217±18
Normal (Treated with pyridoxine for 1 week)	1.85±0.12*	150±16*
Pyridoxine-deficient	0.61±0.19	306±21
Pyridoxine-deficient (Treated with pyridoxine for 1 week)	1.08±0.15*	176±11*

Data are mean±SEM, obtained from eight separate animals in each group

* $P<0.05$ with respect to normal and pyridoxine-deficient respectively (pyridoxine dose, 10 mg/kg i.p. for 7 days)

pyridoxine (10 mg/kg body weight, i.p. daily for the last 7 days). The serotonin content of the cerebral cortex and the B_{max} of serotonin S_2 receptor were determined as described. The results given in Table 3 indicate that pyridoxine deficiency in the pups results in low serotonin levels as compared with normal controls or the deficient pups treated with pyridoxine. Treatment of normal pups with pyridoxine resulted in an augmentation of the serotonin content of cerebral cortex. That the serotonin levels reflect their intrasynaptic release is indicated by the response of the B_{max} of serotonin S_2 receptor binding to its ligand. The results indicate that the pyridoxal phosphate level in regions of the brain regulates the extent of decarboxylation of the 5HTP, the precursor of serotonin.

Discussion

The crucial role played by pyridoxine in the nervous system is evident from the fact that the putative neurotransmitters dopamine (DA), norepinepherin (NE), serotonin (5HT), gamma-amino butyric acid (GABA) and taurine are the products of pyridoxal phosphate (PLP)-dependent enzymatic decarboxylation [4, 6]. There is considerable variation in the affinities of the various apodecarboxylases for PLP which explains the observed differential susceptibility of various PLP enzymes to decrease during progression of pyridoxine deficiency. The synthesis of DA and 5HT is generally considered to be catalyzed by the enzyme aromatic amino acid decarboxylase (EC 4.1.1.28) which lacks substrate specificity. If this decarboxylase were a single protein entity, one would expect parallel decreases of the catecholamines and 5-HT in the tissues of pyridoxine-deficient animals. We have reported [2] nonparallel changes in brain monamines in pyridoxine deficiency. Only 5HT was decreased whereas DA and NE were not affected in pyridoxine deficiency. This is in keeping with the higher affinity of dihydroxyphenylalanine (DOPA) decarboxylase as compared with 5-hydroxytryptophan (5-HTP) decarboxylase for PLP [14]. The decrease in serotonin in pyridoxine deficiency is functionally significant, affecting deep body temperature and sleep [4]. The decrease in hypothalamic 5HT in pyridoxine-deficient rats affects the hypothalamopituitary-end organ axes [3, 5, 6]. A similar decrease in 5HT in the pineal results in decreased melatonin synthesis and pineal function [15]. That the decrease in serotonin in pyridoxine-deficient rats is neuronal in location is indicated by the fact that there was a significant increase in the B_{max} of serotonin S_1 and S_2 receptors in crude synaptic membrane preparations from cerebral cortex of deficient rats [12].

From this neurochemical basis, we suggest that chronic administration of pyridoxine produces an effect on neurotransmitters which is on the other side of the spectrum as compared with pyridoxine deficiency. The neurotransmitter profile resulting from treatment with pyridoxine is duplicated by similar treatment of the animal with a B-vitamin mixture containing pyridoxine, thiamin and vitamin B_{12}. Our results suggest that there is a continuum from deficiency in pyridoxine to treatment of animals with a moderate excess of pyridoxine which is reflected in the synthesis and secretion into the synaptic cleft of the neurotransmitter serotonin.

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References

1. Bartoszyk GD, Wild A (1989) B-Vitamins potentiate the anti-nociceptive effects of diclofenac in carrageenin-induced hyperalgesia in the rat tail pressure test. Neurosci Lett 101:95–100
2. Dakshinamurti K, Leblancq WD, Herchl R, Havlicek V (1976) Nonparallel changes in brain monamines of pyridoxine-deficient growing rats. Exp Brain Res 26:255–366
3. Dakshinamurti K, Paulose CS, Thliveris JA, Vriend J (1985) Thyroid function in pyridoxine-deficient young rats. J Endocrinol 104:339–344
4. Dakshinamurti K (1982) Neurobiology of pyridoxine. In: Draper HH (ed) Advances in nutritional research, vol 4. Plenum Press, New York, pp 143–179
5. Dakshinamurti K, Paulose CS, Vriend J (1986) Hypothyroidism of hypothalamic origin in pyridoxine-deficient rats. J Endocrinol 109:345–349

6. Dakshinamurti K, Paulose CS, Viswanathan M, Siow YL (1988) Neuroendocrinology of pyridoxine deficiency. Neurosci Biobehav Rev 12:189–193
7. Glowinski J, Iversen LI (1966) Regional studies of catecholamines in the rat brain. The disposition of (^{3}H)norepinephrin, (^{3}H)dopamine and (^{3}H)-DOPA in various regions of the brain. J Neurochem 13:655–669
8. Jurna I, Bonke D (1988) Depression of nociceptive activity evoked in the rat thalamus by vitamin B-complex and vitamin B_6. Soc Neurosci Abstr 14:324 (Abstract 134.8)
9. Kilpatrick IC, Jones MW, Phillipson OT (1986) A semiautomatic analysis method for catecholamines, indolamines and semiprominent metabolites in microdissected regions of the nervous system: an isocratic HPLC technique employing caulometric detection and minimal sample preparation. J Neurochem 46:1865–1876
10. Leysen JE, Niemegeers CJE, Van Nueten JM, Laduron PM (1982) (^{3}H)-Ketanserin (R1468), a selective ^{3}H-ligand for serotonin$_2$ receptor binding sites. Mol Pharmacol 21:301–314
11. Murphy RM, Zelman FP (1987) Differential effects of substance-P on serotonin-modulated spinal nociceptive reflexes. Psychopharmacology 93:118–121
12. Paulose CS, Dakshinamurti K (1985) Effect of pyridoxine deficiency on young rats on high affinity serotonin and dopamine receptor. J Neurosci Res 12:263–270
13. Scatchard T (1949) The attractions of proteins for small molecules and ions. Ann NY Acad Sci 51:660–672
14. Siow YL, Dakshinamurti K (1985) Effect of pyridoxine deficiency on aromatic L-amino acid decarboxylase in adult rat brain. Exp Brain Res 59:575–581
15. Viswanathan M, Siow YL, Paulose CS, Dakshinamurti K (1988) Pineal indolamine metabolism in pyridoxine-deficient rat. Brain Res 473:37–42

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