

EFFECT OF PYRIDOXINE DEFICIENCY ON THE STRUCTURAL AND FUNCTIONAL DEVELOPMENT OF HIPPOCAMPUS

A. P. KRISHNA* AND T. RAMAKRISHNA**

**Department of Physiology,
K. S. Hegde Medical Academy,
Nithyananda Nagar, Deralakatte, Mangalore – 575 018*

and

***Department of Life Sciences,
University of Calicut,
Calicut – 673 635*

(Received on December 13, 2003)

Abstract : In this study it was attempted to understand the effect of pyridoxine deficiency on the structural and functional development of the hippocampus. Hippocampus has been closely associated with complex neuroendocrine control of physiological activities as well as behavioural responses including learning process and memory retention. Prenatal, preweaning and weaning deficiency of pyridoxine was induced in the experimental rats by feeding dams with diet deficient in pyridoxine during pregnancy and lactation. The general growth profile for pyridoxine deficient (PD) rats is compared with control ones. The structural changes in the hippocampus of pyridoxine deficient rats was investigated using the histological techniques. Hippocampal electrical activity was recorded from in vitro brain slice preparation. The study clearly showed the structural impairment in the hippocampus of PD rats. These anatomic anomalies might be related to poor neurointegrative development and neurophysiological deficits that occur in young one. The electrical activity recorded from hippocampal slices of PD rats showed significant variation when compared to controls. Pyridoxine deficiency is common in pregnant women who used anovulatory steroids before pregnancy. The pyridoxine deficiency of the mother may result in permanent behavioural abnormality and intellectual deficit in the progeny.

Key words : pyridoxine development hippocampus

INTRODUCTION

Malnutrition in early life has an adverse effect on the growth of body and brain.

Brain is more vulnerable to nutritional insult during its period of growth spurt. Even though informations are available regarding the effect of gross malnutrition

on growth and development of brain and behaviour very little is known about the effect of specific vitamin deficiency. Attempt to study the adverse effect of pyridoxine deficiency in growing animals were made for behavioural and neuromotor parameters (1, 2). In this present study it was attempted to understand the effect of pyridoxine deficiency on the structural and functional development of an important area of brain Hippocampus.

METHODS

Albino rats of Wistar strain were reared with 12h light and 12 h darkness. Food was prepared from Bengal gram flour with vitamin and mineral supplements (3) and was available ad libitum along with water. Rats were divided into control and experimental groups, control pups were reared by the mother fed with normal food containing all the minerals and vitamins in required quantity. Control group animals received 2.5 mg of vitamin per kilogram of diet (3). Experimental pups were reared by the mothers fed with food deprived of pyridoxine, resulting in prenatal and preweaning pyridoxine deficiency. (4) Mothers were fed with pyridoxine deficient diet during pregnancy and lactation. Body weight of rats from the two groups was recorded. Male rats (n = 20) taken from 3 litters of control group and male rats (n = 20) taken from 3 litters of pyridoxine deficient groups were chosen. To assess the growth of the brain, the wet weight of the brain at different stages of development was recorded.

The total DNA content in the hippocampus was estimated in control and pyridoxine

deficient rats of different ages 0, 1, 17 and 20 days. (5). The brain tissue was chopped into small fragments. Then it was homogenised with 200 ml of buffered saline for 1 minute (0.15 mol/L NaCl buffered with 0.015 mol/L citrate, pH7). The suspension was centrifuged at 5000 g for 15 min. and the precipitate was rehomogenised in a further 200 ml of buffered saline. The supernatant was discarded and the combined sediment was suspended in 2 mol/L NaCl to a final volume of 1 L. Sedimentation if any was removed by centrifugation. The solution was stirred continuously with a glass rod while adding an equal volume of distilled water. The fibrous precipitate was spooled on to a glass rod and left to stand in a beaker for 30 minutes.

The deoxyribonucleoprotein was dissolved in about 100 ml of 2 mol/L NaCl and equal volume of chloroform/ amylalcohol mixture (6:1) was added and blended for 30s. The emulsion was centrifuged at 5000 g for 10–15 min. The upper opalescent aqueous layer containing DNA was collected.

Treatment with organic solvent was repeated twice. DNA was precipitated, by slowly stirring 2 volume of ice-cold ethanol with the supernatant. The mass of fibres was collected on the glass stirring rod. The glass rod was removed and the fibrous DNA was pressed gently against the side of the beaker to expel the solvent. Finally the precipitate was washed by dipping the rod into a series of solvents and expelling the solvents. Four solvents were used 70% v/v ethanol, 80% v/v ethanol, absolute ethanol and ether. The last traces of ethanol were

removed by standing the DNA in a fume cupboard for about 10 min.

The dry DNA was weighed. The dilution of DNA was noted. To one ml of DNA solution 4 ml of diphenylamine reagent (Dissolve 10 gm of pure diphenylamine in 1 litre of glacial acetic acid and add 25 ml of concentrated sulphuric acid) was added. It was heated on a boiling water bath for 10 min. and cooled. The extinction was read at 595 nm using a colorimeter. The OD for test and standard was read against a water blank. From the OD of test and OD of standard DNA content was calculated.

Histological sections of hippocampus were taken and stained by silver impregnation techniques as well as Eosin and Hematoxyline. Micro photographs of stained sections were taken using Reichert-Jung (Austria) microscope with camera attachment. The dendritic spine density was determined in the hippocampal sections of control and pyridoxine deficient rats of the postnatal age 10, 15, 20 and 30 days. Six neurons were selected at random from an identical area (CA1) of hippocampus. The spine densities for the 6 neurons were averaged. The calculated averages of spine density was given as the number of spine per micron of dendritic length. Electrophysiological investigation was carried out by recording the electrical activity, from the hippocampal slices. The electrical activity of the hippocampal slices was recorded on a four channel polygraph (Nihon Kohden, Japan). The electrical potential were amplified using preamplifiers. The statistical evaluation of the results were carried out using students 't' test.

RESULTS

The body weight of PD rats of all the ages studied were significantly lower when compared to that of control rats ($P < 0.01$) and this difference increased with age (Fig. 1). The whole brain weight (wet) of vitamin deprived rats was lower when compared to the control group (Fig. 2). The

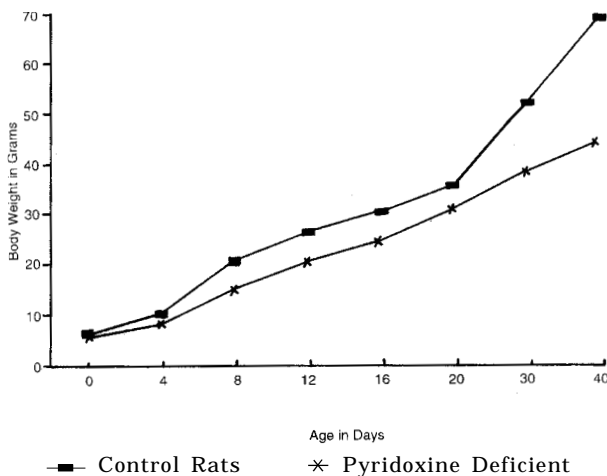


Fig. 1 : Body weight of control and pyridoxine deficient rats.
n = 20; $P > 0.01$

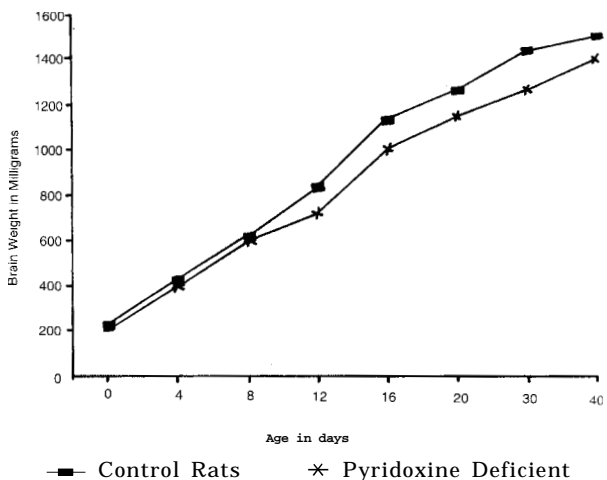


Fig. 2 : Brain weight (wet) of control and pyridoxine deficient rats.
n = 6; $P > 0.01$.

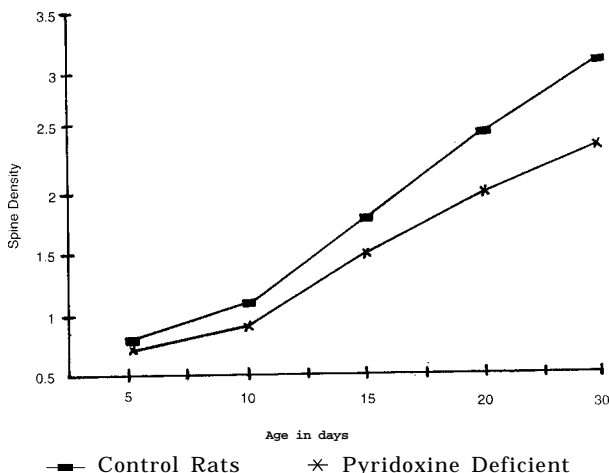


Fig. 3: Dendritic spine density of hippocampus of control and PD rats. n = 6; *P>0.01.

total DNA content in the hippocampus of PD rats at all the ages were less when compared to the controls (Table I). The Microscopic observation indicates, that length of neuronal process was significantly lower in PD rats at all the ages studied, significantly lower at the age of 20 and 30 days (Fig. 3). The recording of electrical activity from the hippocampal slices of control and pyridoxine deficient rats of ages 10, 15, 20, 30 and 40 days were made. The important finding was that the theta

TABLE I: Total DNA content in hippocampus of control and pyridoxine deficient rats.

Age in days	DNA content in micrograms	
	Control rats (n = 6)	Pyridoxine deficient rats (n = 6)
0	86.0±7.85	74.6±4.27*
14	100.33±4.71	92.0±5.53**
17	124.33±6.67	106.66±9.66*
21	126.0±8.66	108.16±10.46**

Values are mean±SD; *P<0.01; **P<0.05.

TABLE II: Mean frequency of high and low amplitude electrical activity of hippocampal slices of control and pyridoxine deficient rats.

Age in days	Mean frequency of electrical activity per minute			
	Control (n = 6)		Pyridoxine deficient (n = 6)	
	< 5 uv	> 5 uv	< 5 uv	> 5 uv
10	12±1	4±1	14±2	2±1*
15	15±2	2±1	14±2	0*
20	5±1	6±2	7±2	4±1
25	15±3	2±1	7±1*	1±1
30	18±3	2±1	11±2*	2±1
40	18±3	6±1	14±2*	11±2*

Values are mean±SD; *P<0.05.

TABLE III: Mean amplitude range of electrical activity hippocampal slices of control and pyridoxine deficient rats.

Age in days	Mean amplitude range in micro volt	
	Control (n = 6)	Pyridoxine deficient (n = 6)
10	2-10	2-7*
15	2-6	2-5
20	2-8	2-7
25	2-7	2-6
30	2-12	2-5
40	2-12	2-9

Values are mean±SD; *P<0.05. Standard deviations are not shown in the table to avoid overcrowding.

rhythm can be recorded from the control rat hippocampal slice at the age of 7.3 days (± 1 day). But the theta rhythm appeared only at the age of 10.3 days (± 1 day) in the PD rats. The adult pattern of theta rhythm was seen at 15 days for control rats and 20 days for PD rats. The mean frequency of electrical activity per minute from HIP slices for control and PD rats is given in the Table II and the amplitude range is given in the Table III.

DISCUSSION

The body weight of PD rats at birth is significantly lower when compared to the control group. The low birth weight of animals is one of the indices of nutritional influences on prenatal development. The low body weight of PD pups is partly related to the depleted fat deposits of pyridoxine deficient mothers (6) which may cause in them poor nursing ability (7) and poor suckling ability of the PD pups (8). The pups of pyridoxine deficient mothers gained weight more slowly than normal pups even when fed by non-deprived foster mothers. This suggests that the prenatal deprivation of pyridoxine, independently affects growth. One of the parameters of growth and development of the CNS is the brain weight at different postnatal ages. The whole brain weight (wet) of PD rats was lower at all the ages studied. At birth the total brain weight of PD rats was only 88.13% of the same in control rats. And this difference in the brain weight between PD and control rats persisted. This result agrees with the earlier studies of Gorziak and Kirksey (9). The low brain weight may be due to lower number of neurons present and the total DNA content in one of the important structures of brain Hippocampus is taken as an index of total number of neurons present in the HIP. The total DNA content of hippocampus of PD rats at birth was only 86.74% of control and this difference persisted. A spurt in brain weight observed during the critical period of growth namely 10 to 20 days of age is associated with the neuron growth and multiplication. The neuron multiplication rate in PD rats were far below the rate in control rats.

Our conceptions of how malnutrition endured early in life affects brain development have evolved and changed considerably. We now know that most of the alterations in the growth of various brain structures eventually recover to some extent. But permanent alternations in hippocampus and cerebellum remain. The age range of vulnerability to these long term effects of malnutrition is very significant (10).

The microscopic observations showed that there were certain structural variations in the hippocampus of PD rats, as evident in terms of shorter and lesser branching of neuronal processes and smaller number of dendritic spines in PD rats when compared to the control of same age group. Similar effects reported by Cordero et al (11), Gundappa and Desiraju (12), Murthy and Desiraju (13). It is postulated that the vulnerable period for this dendritic development is the suckling period.

Medvedev et al (14) have showed that protein calorie deficiency in early postnatal period delayed the time of synaptic arborization. The studies of Grojiak and Kirksey (9) and Kirksey et al (15) partly explained how pyridoxine deficiency can produce such an effect on the synaptic organization in the brain and reduces the number of myelinated axons.

Benitez-Bribiesca L et al (16) have shown that there were severe morphologic abnormalities in the dendritic spines of infants dying of severe malnutrition. These anatomical anomalies might be related to the neurophysiological deficits that occur in these children.

It is being increasingly realized that nervous function depends more on the nervous circuiting than on cell numbers alone. The structural and chemical changes due to pyridoxine deficiency may manifest as behavioural alterations. This can be investigated by the recording of electrical activity from a behaviourally important area of brain-hippocampus. The electrical activity recorded from the HIP slices of PD rats varied from control rats in terms of amplitude, maturation and onset and stabilization of theta rhythm. Nutritional effect on electrical activities of brain was reported earlier by several workers including Stewart et al (1), Rajanna et al (17) and Buckmaster et al (18). How pyridoxine deficiency may alter the electrical activity of the brain is still not clear. Slowed axonal conduction velocity secondary to defective myelination may be one of the causative factors for abnormal electrical activity in pyridoxine deprivation (18). The excitability of the CNS may be increased possibly, due to decreased synthesis of inhibiting neurotransmitter

GABA (γ -aminobutyric acid)-Kurlmann et al (19). It was also established that pyridoxine deficiency leads to change in brain concentration of serotonin (20).

The adverse effect brought about by the pyridoxine deficiency on the development of CNS emphasizes the importance of pyridoxine as a nutritional factor during pregnancy. Anovulatory steroids are known to induce pyridoxine deficiency (21) and women who use contraceptive pills for prolonged period of time may have a relative deficiency of pyridoxine (22). To ensure the normal development of the child, maternal pyridoxine supplementation during pregnancy and lactation is recommended.

ACKNOWLEDGEMENTS

The first author is grateful to Dr. M. Shantharam Shetty, Dean, K. S. Hegde Medical Academy, Mangalore and to Sri N. Vinay Hegde President, Nitte Education Trust, Mangalore for their encouragement and support.

REFERENCES

1. Stewart CN, Coursin DB, Bhagawan HN. Avoidance behaviour in vitamin B6 Deficient rats. *J Nutr* 1975; 106: 1363-1380.
2. Krishna AP, Ramakrishna T. Effect of Pyridoxine deficiency on the development of neuromotor coordination's *Indian J Physiol Pharmacol* 1994; 38(2): 113-116.
3. Ford D. Diets (natural and synthetic) Rats and Mice. In *CRC Handbook in Nutrition and Food* (ED) Rechiegal M. Jr. C.R.C. Press 1977; 107-132.
4. Lakshmi AV, Diwan PV, Bamji MS. Wound healing in pyridoxine deficiency. *Nutrition Research* 1988; Vol. 8: 1203-1206.
5. Plummer DT. An introduction to practical Biochemistry Tata Mcgraw Hill Publishing Company Ltd, New Delhi 1987; 216-222.
6. Mc Henry E, Wand Gavin G. The B vitamins and fat metabolism (a) effect of thiamine, riboflavin and rice polish concentrate upon body. *Fat J Biol Chem* 1938; 125: 653-658.
7. SaboDJ, Franscesconi RP. Gershoff SN. Effect of vitamin B6 deficiency on tissue dehydrogenase and fat synthesis in rat. *J Nutr* 1971; 101: 29-34.
8. Alton-Mackey MG, Walker BL. Graded levels of pyridoxine in the rat diet during gestation and the physical and the neuromotor development of offspring. *Amer J Clin Nutr* 1973; 26: 420-428.

9. Gorziak SM, Kirksey A. Effects of maternal dietary restrictions in vitamin B6 on neocortex development in rats B6 – vitamin concentrations, volume and cell estimates *J Nutr* 1987; 117(6): 1045–1052.
10. Levitsky DA, Strupp BJ. Malnutrition and Brain: Changing concepts, changing concerns. *J Nutrition* 1995; 125(8): 2212s–2220s.
11. Cordero ME, TrejoM, Goreia E, Barros T, Rojan AM, Colomb M Dendritic development in the neocortex of adult rats following a prenatal and early postnatal life undernutrition. *Early Hum Dev* 1986; 14(3–4): 245–258.
12. Gundappa, Desiraju T. Deviations in brain development of F2 generation on caloric undernutrition and scope of their prevention by rehabilitation. Alterations in dendritic spine production and pruning pyramidal neurons of lower laminal of motor cortex and visual cortex. *Brain Research* 1988; 456: 205–223.
13. Murthy KD Desiraju T. synapses in developing cingulated and hippocampal cortices in undernourished rats. *Neuro Report* 1991; 2 No. 8: 433–436.
14. Medvedev DI, Babichenko. II, Karavtsova AI, Eremina. Effect of protein calorie deficiency on the ultra structure of synapses in the molecular layer of the cerebellar cortex in developing and adult animals *Biull Eksp. Biol Med* 1988; 106(7): 106–108.
15. Kirksey A, Morre DM, Wasyncjuk. Neuronal development in vitamin B6 deficiency. *Ann NY Acad Sci* 1990; 585: 202–218.
16. Benitez-Bribiesca L et al Dendritic spine pathology in infants with severe protein calorie malnutrition. *Paediatrics* 1999; 104(2): 21.
17. Rajanna B, Masarenhas, Desiraju T. Deviation in brain development due to caloric undernutrition and scope of their prevention by rehabilitation- Alterations in the power spectra of EEG of areas of the neocortex and limbic system. *Brain Res* 1987; 465: 97–113.
18. Buckmaster PS, Holiday TA, Bai SC, Rogers QR. Brain Stem evoked potential interwave intervals are prolonged in vitamin B6-deficient cats. *J Nutr* 1993; 123(1): 20–26.
19. Kurlemann G, Loscher W, Dominick HC, Palm GD. Disappearance of neonatal seizures and low C.S.F. GABA levels after treatment with vitamin B6. *Epilepsy Res* 1987; 1(2): 152–154.
20. Dakshinamoorthi K, Sharma SK, Banke D. Influence of B vitamins on binding properties of serotonin receptor in the C.N.S. of rats. *Klin Wochenscher* 1989; 19: 68(2): 142–145.
21. Rose DP. The influence of estrogen or tryptophan metabolism in man. *Clin Sci* 1966; 31: 265–270.
22. Wachstein M. Evidence for a relative vitamin B6 deficiency in pregnancy and some diseased states. *Vitamins and Hormones* 1964; 22: 705–719.