International Journal of Anatomy and Physiology ISSN: 2326-7275 Vol. 6 (12), pp. 001-003, December, 2017. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Short Communication

Effects of vitamin B6 on age associated changes of rat brain glutamate decarboxylase activity

Manoochehr Messripour[1]* and Azadeh Mesripour[2]

[1] Department of Clinical Biochemistry, Khorasgan Branch, Islamic Azad University, Esfahan, Iran
[2]Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran.

Accepted 15 April, 2017

Glutamate and gamma-aminobutyric acid (GABA), act as excitatory and inhibitory neurotransmitters in CNS respectively. An increase in glutamate and a decrease in GABA concentration were observed in aged brain. However, the mechanism of these changes has not been very well elucidated. Glutamate decarboxylase (GAD) catalyzes the conversion of glutamate to GABA. Since the vitamin B6 is essential for the activities of GAD, this study was undertaken to investigate the effects of vitamin B6 administration on age related changes in rat brain. The animals were injected intraperitoneally with 1, 10 and 100 mg vitamin B6 /kg body weight /day for 30 days, and specific activity of GAD was assayed in the brain supernatant. The activity of the enzyme in aged rats was significantly lower as compared to that of young animals. Vitamin B6 induced activation of the brain enzyme in both ages, but the rate of the activation was markedly pronounced in aged animals. Significant activation rate of GAD by vitamin B6 in aged rat brain may be resulted from either lower availability of vitamin B6 in aged animals, or lower affinity of the enzyme for pyridoxal -5-phosphate, which is likely to be related to conformational changes of the enzyme during aging. It is suggested that vitamin B6 may restore the activity of the brain glutamate decarboxylase in aged rat.

Key words: Glutamate, gamma-aminobutyric acid, vitamin B6, aging, glutamate decarboxylase.

INTRODUCTION

Age associated changes in the brain metabolism of neurotransmitters are thought to be important and could possibly lead to many age-related symptoms. Many changes have been reported to occur in glutamatergic and GABAergic, transmitter systems in aging (Hardy et al., 1985; Reinikainen et al., 1988; Lowe et al., 1988) . In the aged postmortem brain regions an increase in glutamate concentration and a decrease in GABA concentration were observed. Glutamate is the major excitatory neurotransmitter in CNS, which is involved in several synaptic plasticities such as long-term ostentation and depression, and is also believed to underlie learning and memory (Hardy et al., 1985; Reinikainen et al., 1988; lowe et al., 1988). Because almost no glutamate crosses from blood to brain, it must be synthesized in the presynaptic regions of the neurons, and released into the synaptic cleft, by a Ca^{2+}-dependent process that involves that involves fusion of glutamate-containing presynaptic vesicles with the neuronal membrane (Daniel and Daniel, 2008). Excessive levels of extracellular glutamate in the brain are known to be excitotoxic and lead to neuronal death (Daniel and Daniel, 2008; Corlew et al., 2008). Furthermore, it has been suggested that the glutamate receptor antagonists can be used as the therapeutic commands in Alzheimer's disease (Shevtsov et al., 2008). It is however, reasonable to assume that rapid removal of the released glutamate in the synapses may also prevent the excessive excitation of glutamate receptors. Moreover, GABA is known to act as an inhibitory neurotransmitter in the brain and the enzyme glutamate decarboxylase (GAD) plays an important role in the balance of glutamate/GABA concentrations in the brain (Kure et al., 1998; Milton et al., 2002). The vitamin B6 derivative pyridoxal phosphate is a cofactor in the synthesis of GABA, which the resultant GABA inhibitory effects play an important role in the protection of the brain neurons (Kure et al., 1998; Gospe et al., 1994). Since vitamin B6 is reported to slow the accelerated rate

*Corresponding author. E-mail: m_messripour@yahoo.com. Tel: 0098 311 5354059. Fax: 0098 311 5354060.

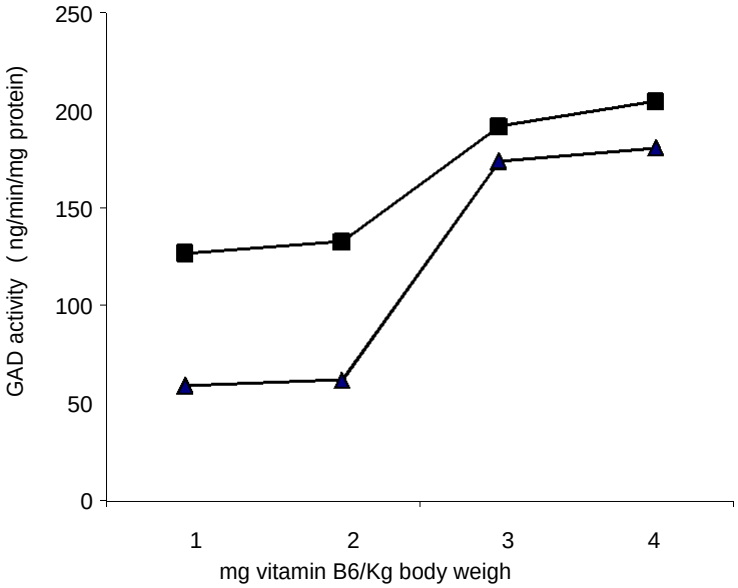

Figure 1. The effect of vitamin B6 on the activity of GAD in young and old rats.Three months ( ) and 30 months old( ) rats were injected intraperitoneally with 0 (control), 1 (2), 10 (3) and 100 (4) mg vitamin B6 /kg body weight /day for a period of 30 days and GAD activity was measured in the brain supernatant. Each point is mean of 6 separate experiments with SD less than 10%. Differences between young and old rats at points 1 and 2 are statistically significant; ($p<0.05$), and at points of 3 and 4 are insignificant.

of brain atrophy in elderly with mild cognitive impairment (Sun et al., 2007), the present study was designated to determine the effect of vitamin B6 administration on GAD activity in young and aged rat brain.

MATERIALS AND METHODS

Animals and treatments

Twenty four young (3 months old) male Wistar rats with weight ranging from 200 to 250 g and 24 old (30 months old) male Wistar rats (30 months old) with weight ranging from 650 to 720 g were used. Animals were maintained with respect to the animal welfare regulation in animal house until the desired age was attained. The animals were injected intraperitoneally with 1, 10 and 100 mg vitamin B6 /kg body weight /day for a period of 30 days. Control group were injected with saline only. The control, and experimental groups were housed (6 rats in each group) in a regulated environment (25±1°C; 50 to 55% relative humidity; 12 h light/dark cycle), with free access to food and water. The day after last injection the animals were killed by decapitation after anesthesia. The animal brains were removed immediately and homogenized in phosphate buffer (pH 7) at 4°C and centrifuged at 70, 000 g.

The activity of GAD was measured in the brain supernatant spectrophotometrically as described by Cozzani (1970). Briefly, the brain supernatant (1 ml) was adjusted to pH 7 and transferred in the cuvet and incubated at 37°C for 5 min. The reaction was then started by addition of 100 l glutamate solution (10 mM) and decarboxylation of glutamate at 340 nm was monitored against a blank containing all components except glutamate. The rate of glutamate decarboxylation was linear up to 30 min. The determination of the enzyme protein concentration was performed by the method of Lowry et al (1951). Significance level was set at $p<0.05$, using Student's t-test.

RESULTS

The activity of GAD in aged rats brain was 54% lower than that of young animals ($p<0.05$) . The effects of vitamin B6 administration in doses of 0 to 100 mg/kg body weight on the activity of the brain GAD of young and old rats is shown in the Figure 1. The enzyme activity was activated by administration of vitamin B6 in a non linear fashion in both ages, although the activation was more pronounced in aged animals ($p<0.05$) Activation of the enzyme was augmented markedly with doses of 10 mg/kg body weight, whereas doses higher than 10 mg/kg body weight did not increase the enzyme activity significantly.

DISCUSSION

The first part of this *in vivo* study sought to measure GAD specific activities in the brain as a function of the age of the rats. The results indicate that the activities of the enzyme are significantly lower in the brain of aged rats as compare to that of young animals. Lower activity of GAD in the brain of aged animals may alter the balance of glutamate and GABA concentration in favor of glutamate

accumulations in the synaptic parts of certain brain regions. This suggestion is interpreted as being consisted with the hyperactivity of glutamatergic system in aged brain (Hoop et al., 1990; Thomas, 1995). Activation of GAD by vitamin B6 indicates that pyridoxal-5- phosphate which is an essential part of the active site of the enzyme might be at suboptimal levels. However, the rate of activation was considerably greater in the aged rat brain as compared to that in young animals. Previous reports focusing on the effects of vitamin B6 deficiency on rat brain GAD demonstrated that level of the GAD apoenzyme was higher in the deficient young rats but not in aged animals (Driskell and Chuang, 1974; Rajeswari and Radha, 1984). It appears that GAD apoenzyme could not be induced in old animals as a function of B6 deficiency. Although, provided data is not adequate to suggest about the interaction of prydoxal-5-phosphate with the enzyme at the molecular levels, it seems that the significant activation of the enzyme in aged rat brain with vitamin B6, might be resulted from either; higher GAD apoenzyme level in aged animals (Rajeswari and Radha, 1984), or lower affinity of the enzyme for pyridoxal 5'-phosphate. The latter is likely to be related to the post-translational modifications of the proteins as consequences of aging (Messripour et al., 1994). This is consistent with the results reported from the treatment of individuals with mild to moderate cognitive disorders with B vitamin supplements (Sun et al., 2007). Although, GAD might be considered as a therapeutic target for prevention of neurodegenerative disorders and age related symptoms, it seems unlikely to improve Alzheimer's disease symptoms. It is however, concluded that restoring GAD activity in aged animals by vitamin B6 to the levels equivalent to young animals might prevent the glutamate neurotoxicity during aging.

REFERENCES

Corlew R, Brasier DJ, Feldman DE (2008). Presynaptic NMDA receptors: newly appreciated roles in cortical synaptic function and plasticity. Neuro. Sci., 14(6): 609-625.

Cozzani I (1970). Spectrophotometric assay of -glutamic acid decarboxylase, Anal. Biochem., 33(1): 125-131.

Daniel JB, Daniel EF (2008). Synapse-specific expression of functional presynaptic NMDA receptors in rat somatosensory cortex. J. Neuro. Sci., 27: 28(9): 2199-2211.

Driskell JD, Chuang SL (1974). Relationship between glutamate decarboxylase activities in brains and the vitamin B6 requirement of male rats, J. Nutr., 104: 1657-1661.

Driskell JD, Chuang SL (1974). Relationship between glutamate decarboxylase activities in brains and the vitamin B6 requirement of male rats, J. Nutr., 104: 1657-1661.

Gospe SM Jr, Olin KL, Keen CL (1994). Reduced GABA synthesis in pyridoxine-dependent seizures. Lancet., 343: 1133-1134.

Hardy J, Adolfsson R, Alafuzoff I (1985). Transmitter deficits in Alzheimer's disease, Neurochem Int., 7: 545-563.

Hoop B, Masjedi MR, Shih VE, Kazemi H (1990). Brain glutamate metabolism during hypoxia and peripheral chemodenervation. J. Appl. Physiol., 69: 147-154.

Kure S, Sakata Y, Miyabayash S (1998). Mutation and polymorphic marker analyses of 65K- and 67-glutamate decarboxylase genes in two families with pyridoxine-dependent epilepsy. J. Hum. Genet., 43(2): 128-131.

Lowe SL, Francis PT, Procter AW (1988). Gamma-aminobutyric acid concentration in brain tissue at two stages of Alzheimer's disease. Brain, 111: 785-799.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.

Messripour M, Weltin D, Rastegar A (1994). Age-associated changes of rat brain neuronal and astroglial poly (ADP-ribose) polymerase activity. J. Neurochem., 62(2): 502-506.

Milton SL, Thompson JW, Lutz PL (2002). Mechanisms for maintaining extracellular glutamate levels in the anoxic turtle striatum. Am. J. Physiol. Regul. Integr. Comp. Physiol., 282: R1317-R1323.

Rajeswari TS, Radha E (1984). Age-related effects of nutritional vitamin B6 deficiency on B6-dependent enzymes of glutamate, gamma-aminobutyrate and glutamine systems in the rat brain. Exp. Gerontol., 19(2): 87-93.

Reinikainen KJ, Paljarvi L, Huuskonen M (1988). A post -mortem study of noradrenergic, serotonergic and GABAergic neurons in Alzheimer's disease. J. Neurol. Sci., 84: 101-116.

Shevtsov PN, Shevtsova EF, Burbaeva GS (2008). Effect of tacrine, amiridine, akatinol memantine, and triazolam on phosphorylation, structure, and assembly of microtubules from brain microtubular proteins in Alzheimer diseases. Bull. Exp. Biol. Med., 145(2): 218-222.

Sun Y, Lu CJ, Chien KL (2007). Efficacy of multivitamin supplementation containing vitamins B6 and B12 and folic acid as adjunctive treatment with a cholinesterase inhibitor in Alzheimer's disease: a 26-week, randomized, double-blind, placebo-controlled study in Taiwanese patients. Clin. Clin. Ther., 29(10): 2204-2214.

Thomas RJ (1995). Excitatory amino acids in health and disease. J. Am. Geriatrics Soc., 43(11): 1279-1289.