Research Article

Anupama Sharma* and Renu Bist

Alteration in cholinesterases, γ -aminobutyric acid and serotonin level with respect to thiamine deficiency in Swiss mice

Tiamin Eksikliğine olan farelerde Kolinesteraz, γ -aminobutirik asit ve Serotonin Seviyesinde Değişim

<https://doi.org/10.1515/tjb-2017-0316>

Received November 15, 2017; accepted April 3, 2018; previously published online August 28, 2018

Abstract: Thiamine (vitamin B1), cofactor for various multi-enzyme complexes in energy metabolism, and plays a major role in the synthesis of cholinesterases such as acetylcholinesterase (AChE); butyrylcholinesterase (BChE). Present study deals with the changes in the cholinesterases, γ -aminobutyric acid (GABA) and serotonin in mice brain following thiamine deficiency. Experimental mice (6–8 week old) were made thiamine deficient by intraperitoneal injection of pyriethamine hydrobromide and fed with thiamine-deficient diet. Animals were divided into three groups, Group I (Control), Group II (thiamine deficient mice for 8 days), and Group III (thiamine deficient mice for 10 days). The higher serotonin level whereas significant decreases in the AChE, BChE and GABA level were recorded in treated groups as compared to control. Hence, vitamin B1 deficiency disturbs the cholinergic system and neurotransmitters levels in brain which may lead to neurodegenerative diseases.

Keywords: AChE; BChE; GABA; Vitamin B1.

Öz: Tiamin (vitamin B1), enerji metabolizmasında çeşitli multi-enzim kompleksleri için kofaktör ve

Asetilkolinesteraz (AChE), Butirilkolinesteraz (BChE) gibi kolinesterazların sentezinde önemli bir rol oynar; Bu çalışma tiamin eksikliğini takiben farelerde beyindeki kolinesteraz, γ -aminobütirik asit (GABA) ve serotonin değişikliklerini ele almaktadır. Deney fareleri (6–8 haftalık) intraperitoneal pitmiamin hidrobromür enjeksiyonu ile tiyamin eksikliği yapıldı ve tiamin eksikliği olan diyetle beslendi. Hayvanlar 3 gruba ayrıldı, Grup I (Kontrol), Grup II (8 gün boyunca tiamin eksikliği olan fareler) ve Grup III (10 gün boyunca tiamin eksikli fareler). Kontrol grubuna kıyasla tedavi edilen gruplarda daha yüksek serotonin seviyesi, AChE, BChE ve GABA seviyesinde önemli düşüşler kaydedildi. Bu nedenle, B1 vitamini eksikliği, nörodejeneratif hastalıklara yol açabilen beyin içindeki kolinerjik sistemi ve nörotransmitter seviyelerini bozabilir.

Anahtar Kelimeler: AChE; BChE; GABA; Vitamin B1.

Introduction

Thiamine, an essential vital water soluble vitamin which is required continuously in daily dietary intake to support as a cofactor for several multi enzyme complex of carbohydrate metabolism [1]. It also plays a major role in the synthesis of acetylcholinesterase (AChE). Breads, fish, legumes, meat and eggs are the sources of thiamine [2]. The recommended dietary intake of thiamine for men and women is in the range of 1–1.5 mg/day approximately. Cholinergic system in brain involved in memory and learning. Any alteration in cholinergic system affects the metabolism of brain and causes neurodegenerative diseases such as Alzheimer's disease [3]. There are two types of cholinergic enzymes namely acetylcholinestrases

*Corresponding author: Anupama Sharma, Banasthali Vidyapith Faculty of Life Sciences Ringgold Standard Institution, Department of Bioscience and Biotechnology, Jaipur, India, e-mail: anupamasharma.biotech@gmail.com. <https://orcid.org/0000-0001-5578-3784>

Renu Bist: Banasthali Vidyapith Faculty of Life Sciences Ringgold Standard Institution, Department of Bioscience and Biotechnology, Jaipur, India

and butyrylcholinesterases (BChE) were found in brain and function of AChE and BChE is to terminate the action of acetylcholine and butyrylcholine in nerve terminals [4]. AChE is a key enzyme plays a important function in nervous system [5]. It catalyzes the acetylcholine at synaptic cleft to transmit the nerve signal from one neuron to another. The activity of AChE and BChE are in with synergistic relationship to maintain the cholinergic function. Therefore, alteration in AChE activity directly affects the level of BChE in the body [6]. Inhibition of AChE and BChE can act as a biomarker for several diseases [7].

In addition, γ -aminobutyric acid (GABA) and serotonin are neurotransmitters found in brain. GABA is a major inhibitory neurotransmitter and produced from glutamic acid. It reduces neuronal excitability which helps in dropping down the anxiety and pain. Serotonin is a biogenic amine and excitatory neurotransmitter of the body. Only 1–2% of the total serotonin is found in brain. It rapidly elevates O_2^- formation in pulmonary artery smooth muscle cells and its metabolites are aptly to enhance plasma lipid peroxidation via changes in the redox potential. Enhancement of serotonin level promotes oxidative stress [8] which is one of the key features of neurodegenerative diseases caused due to thiamine deficiency [1]. Hence, present study was designed to observe the effect of thiamine deficiency on cholinesterases (AChE; BChE), GABA and serotonin level in mice.

Materials and methods

Chemicals

Pyrithiamine hydrobromide and acetylthiocholine iodide were purchased from Sigma (MO, USA). 5,5'-Dithiobis-2-nitrobenzoic acid, (DTNB), butyrylthiocholine iodide, EDTA, tris hydrochloride, trichloroacetic acid (TCA), formaldehyde, ethanol and pure wax were purchased from Sisco Research Laboratories (Mumbai, India). All other chemicals were of analytical grade.

Animal care and monitoring

Healthy *Mus musculus* (6–8 week old) were procured from C.C.S. Haryana Agricultural University, Hissar. They were fed with pelleted diet (Hindustan Uniliver Limited, Mumbai, India) and water ad libitum. After 3–7 days of familiarization, mice were used for experimental purpose. Maintenance and treatment of animal were done in accordance with the Committee for the Purpose of

Control and Supervision of Experimentation on Animals (CPCSEA).

Experimental design

The animals were divided into three groups having six animals in each group.

Group I Normal pelleted diet + normal saline

Group II Thiamine deficient diet + pyrithiamine exposed for 8 days

Group III Thiamine deficient diet + pyrithiamine exposed for 10 days

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Induction of thiamine deficiency (TD)

Mice were made thiamine deficient by injecting the pyrithiamine hydrobromide (5 μ g/10 g of body weight) intraperitoneally and fed with thiamine deficient pelleted diet. Control animals were fed with normal diet.

Biochemical studies

Sample preparation for AChE and BChE

After the treatment, experimental mice were sacrificed by cervical dislocation. Brains were removed and washed with normal saline. The tissues were homogenized in the specific buffer (0.1 M phosphate buffer) and centrifuged at 1200 g for 10 min at 4°C. Further, supernatant was used for biochemical analysis of AChE and BChE.

Acetylcholinesterase (AChE)

AChE releases thiocholine from acetylcholine which reacts with the DTNB, reducing it to the thiol which has an absorption maximum at 412 nm. The activity of AChE was assayed by the method of Ellman et al. [9]. Protein was measured by the method given by Lowry et al. [10]. The activity of AChE enzyme was expressed in terms of nmol/mg of protein/min.

Butyrylcholinesterase (BChE)

Ellman et al. [9] method was used for determination of BChE activity. BChE releases thiocholine from

butyrylcholine and reacts with DTNB reduced into thiol which shows maximum absorption at 412 nm. Results were expressed as nmol/mg of protein/min.

γ -Aminobutyric acid (GABA) content

The level of GABA was measured in aliquots of the perchlorate free extract [11]. The reaction mixture contains 400 μ L of extract (sample), 100 μ L of 2-mercaptoethanol, 100 μ L of NADP, 100 μ L of α -ketoglutarate, 250 μ L of buffer and 50 μ L of Gabase. Samples were mixed and read at 340 nm. Results were expressed in terms of μ g GABA/g of tissue weight.

Serotonin level

Brains were removed and washed in normal saline. The tissues (300 mg) were weighed and homogenized in 3 mL of borate buffer (pH 10) saturated with n-butanol. Serotonin was extracted from the tissue. In the extract, 1 mL of nitroso naphthol and 1 mL of nitrous reagent were added. The extract was incubated on waterbath for 5 min at 55°C. Dichloroethane was added in the samples and shaken properly. Samples were centrifuged at 3000 rpm for 10 min at 4°C. Absorbance of the aqueous phase was measured at 540 nm [12]. Results were expressed as of μ g serotonin/g of tissue weight.

Statistical analysis

Results were expressed as mean $\pm$ SE. Data of present investigation was statistically analyzed by one-way analysis of variance (ANOVA) using (SPSS 16.0). Inter group comparison was made by using Tukey's test.

Effect of TD on neurotransmitters level in brain homogenate of *Mus musculus*

Effect on AChE (Figure 1)

Data of the current study showed that significantly lower AChE activity was determined in treated groups which were made thiamine deficient for 8 ($p < 0.05$) and 10 days ($p < 0.0001$) with respect to control group. As compared to group II, decline AChE activity was observed in group III. Among treated and non-treated groups, AChE activity was found to be lower in group III.

Figure 1: AChE levels in brain of different experimental groups. Results were expressed in mean $\pm$ SE. (a) compared to group I; (b) compared to group II; (c) compared to group III. * $p < 0.05$, **** $p < 0.0001$.

Figure 2: BChE levels in brain of different experimental groups. Results were expressed in mean $\pm$ SE. (a) compared to group I; (c) compared to group III. * $p < 0.05$.

Effect on BChE (Figure 2)

As consequences of TD, the activity of BChE was altered in different experimental groups. When compared to control group, decline in BChE activity was recorded in group II. In treated group III, BChE activity was significantly decreased ($p < 0.05$) with respect to control group. Thus, it was explained from Figure 2 that the maximum reduction in BChE activity was found to be in group III with respect to other groups.

Effect on GABA content (Figure 3)

A significant reduction in the level of GABA was observed in group II ($p < 0.05$) and group III ($p < 0.0001$) as compared to group I. In comparison with group II, decreased content of GABA was recorded in group III ($p < 0.01$).

Figure 3: GABA content in brain of different experimental groups. Results were expressed in mean $\pm$ SE. (a) compared to group I; (b) compared to group II; (c) compared to group III. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.

Figure 4: Serotonin content in brain of different experimental groups. Results were expressed in mean $\pm$ SE. (a) compared to group I; (b) compared to group II; (c) compared to group III. **** $p < 0.0001$.

Among all the groups, lower GABA content was observed in the group III

Effect on serotonin level (Figure 4)

Serotonin content was found to be successively increased in treated groups. A significant rise ($p < 0.0001$) in the echelon of serotonin was recorded in treated groups (groups II and III) as compared to control group. Also, significantly higher ($p < 0.0001$) serotonin content was found in group III with respect to group I and group II.

Discussion

Brain is a fascinating director which coordinates and regulates different organ systems in the body. Experimental

TD is a classical model of a nutritional deficit associated with an impairment of oxidative metabolism and selective cell damage in the brain [13]. Thiamine serves as a specific cofactor of certain enzymes involved in energy metabolism of cells. These enzymes play pivotal role in the synthesis of number of cell constituents including neurotransmitters and production of reducing equivalent used in oxidative stress defense system [14].

Thiamine deficiency induces oxidative stress in brain and adversely affects its functions. It can act as a free radical scavenger [1]. In the present study, thiamine deficiency leads to reduction in AChE and BChE activity in brains of mice. Decline in AChE may be explained due to a direct or indirect inhibition of the central and peripheral cholinergic neurotransmitter system by sub-acute and acute deprivation of thiamine [6]. AChE plays a key role in cholinergic neurotransmission [15]. It is an important enzyme which regulates the effects of acetylcholine at cholinergic synapses. Under stress condition developed due to thiamine deficiency, AChE activity is inhibited which further interrupt the acetylcholine cleavage activity. Current study was also supported with previous research carried out by Pires et al. [16] reported diminution in AChE activity in different regions of thiamine deficient brain.

The results of current research showed declined BChE activity in thiamine deficient groups exposed for 8 and 10 days as compared to control. It also inactivates the neurotransmitter acetylcholine and therefore it is a viable therapeutic target against neurological disorders [17]. However, BChE is less efficient in ACh hydrolysis at low amount, but more efficient at higher concentrations [18]. Bist and Bhatt [4] enlightened the spatial relationship of glial BChE, which would permit BChE mediated synergistic hydrolysis of acetylcholine to allow the maintenance of normal cholinergic functions in the brain. In rat brain, lower AChE activity was recorded under stress conditions given by Ramkumar et al. [19] and Shankaranarayana et al. [20]. Hence, a possible role of BChE is when it is associated with glia cells and helps in hydrolysis of acetylcholine. Under the conditions of higher BChE activity in brain, local synaptic acetylcholine can reach at micromolar levels. Current study evaluated the reduced activity of BChE in comparison with AChE activity in brain because it was also seen by previous study that BChE concentration was about one tenth of AChE in brain [21].

Further, GABA plays a chief role in neuronal development and communication. The result of present study showed decreased level of GABA in brain of thiamine deficient mice as compared to control one. This could be due to the inhibition of GAD following the local formation of 2 keto-4-pentenoic acid. Since GABA is a vital inhibitory

neurotransmitter in the CNS, therefore, it possibly may block the transmission of nerve impulse, which prevents over stimulation of the nerve cells. GABA is degraded through the GABA shunt, operating in both neurons and astrocytes to allow the carbon skeleton of GABA to re-enter the TCA cycle as succinate [22, 23]. Therefore, any alteration in TCA cycle affects the level of GABA in brain. Thiamine deficiency alters the activities of TCA cycle enzymes reported by Sharma et al. [24]. Bist and Bhatt [8] observed reduction in GABA content of brain of lindane intoxicated mice. In Schizophrenic patients, reduction of α -ketoglutarate dehydrogenase complex (KGDHC) activity may be related with an increase in the GABA content [25–27]. Batra and Seth [28] elucidated that decrease in GABA under stress may be due to altered metabolism of GABA synthesis. Alteration in GABA metabolism is associated with various neurodegenerative disorders such as epilepsy, algesia, anxiety, Parkinsonism and Huntington's diseases [29, 30]. In Experimental TD in PTD model, decreases in thalamic GABA levels was also reported [31].

Additionally, present study elucidated that deprivation of thiamine perturbs the serotonin level of mice brain. The higher serotonin level was observed in treated groups as compared to control. This could be due to the unavailability of serotonin receptors in brain. As brain has high lipid content, it acts as a chief target of γ -HCH and solubilize the pre and postsynaptic elements. The unavailability of receptors enhances the serotonin concentration in brain which will finally lead to production of free radicals. The increased serotonin level promotes LPO in brain was elucidated previously [8, 32]. Lee et al. [33] noted that increase in serotonin rapidly elevates the level of superoxide radicals in pulmonary artery smooth muscles. 5-HT neurons in thiamine deficient conditions remain intact structurally but function of 5-HT get affected during the progression of thiamine deficiency. This alteration may be part of adaptive neuronal alteration subsequent to thiamine dysfunction which may a leading cause of neurodegenerative diseases [34].

Conclusion

Hence, the current study concluded that thiamine deficiency reduces the activities of AChE and BChE, GABA level whereas higher serotonin level in *Swiss albino* mice which may leads to neurodegenerative disorders.

Acknowledgments: Authors are thankful to Prof. Aditya Shastri, Vice-Chancellor, Banasthali University Rajasthan

for providing the suitable facilities to carry out the present investigation in Department of Bioscience and Biotechnology and Indian council of medical research (ICMR), New Delhi for financial support (Funder Id: 10.13039/501100001411, 2011-11080).

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