Influence of Acute Workouts on the BDNF Levels in Hippocampus-exposed Rats

Mohammad Shahandeh[1]*, Vaghinak Sarkisian[1]

[1] L. Orbeli Institute of Physiology, NAS of Armenia, Yerevan, Armenia

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Abstract

Purpose: It was shown that with acute low-intensity exercise that is minimally stressful, hippocampus activation and BDNF expression can be achieved lending support to the idea that mild exercise could yield greater benefits in hippocampus functions compared to the more strenuous forms. The neuroprotective effects of acute workout training on lead acetate-induced BDNF levels were investigated.

Material and Methods: Thirty -two adult, male, Wistar rats were randomly classified into 4 groups; Rats were randomly assigned to 1) basic group (Base-B, *n* = 10), 2) control group (lead-L, n=10) 3), lead injection group (acute pre, n=10) and 4) treadmill exercise with lead injection group (acute post, n=10). The rats in groups 3 and 4 experienced the treadmill running 15 to 22m/min, 25 to 64minutes, 5 times a week, and for 8 weeks. Groups 3 and 4 received lead acetate (20 mg/kg) and the sham group received solvent (ethyl oleat).

Results: The results showed that BDNF in Lead acetate group decreased significantly whereas it increased in Lead acetate + Workout group. Obtained data suggest a lifestyle-induced protective potential in rehabilitation of lead-induced neurodegeneration.

Discussion and Conclusion: findings from the present research indicated the significant role of exercises in improving BDNF level. Increased level of BDNF may act as neuroprotector in recovery from numerous disturbances, such as reduction of the brain weight, memory loss and different degenerative processes. It may be concluded that the increase of BDNF in hippocampus may cause positive plastic changes and prevent the mentioned disturbance.

Keywords: Acute workout, Brain-derived neurotrophic factor, Hippocampus, Lead acetate

Introduction

It has been suggested that physical exercise modulates cognitive functions through various signaling mechanisms that lead to brain-derived neurotrophic factor (BDNF) up-regulation, especially in the hippocampus, a major hub for learning and memory formation [1,2]. BDNF is needed for appropriate functioning of the brain. Studies on BDNF have shown differences in brain region size, memory functioning and anxiety-related behavior when BDNF expression is altered [3]. It is important for cell survival in neurogenesis studies and has been thought to have an important antidepressant role [5]. This has promoted research on BDNF levels in health and disease states, in the hope of a better understanding of its etiology and curative effects [6].

It is known that lead is one of the most widespread toxicants and its poisoning remains a health threat [7]. Several lines of evidence implicate high-level lead exposure as a cause of many of pathological conditions such as renal insufficiency, gout and hypertension [8]. Lead is a polluting agent of the ecosystem and is introduced into the food chain by various mechanisms. Lead poisoning is, and for centuries has been, one of the most significant preventable causes of neurological morbidity from an environmental toxin. As a heavy metal, lead is ubiquitous in our environment, yet it has no physiologic role in biological systems. Its effects are pervasive and often subtle, with consequences ranging from cognitive impairment in children to peripheral neuropathy in adults.

Animal models provide information on the effects of low- and medium-level lead effects on neural and cognitive processes [9], and for the most part, the animal models and epidemiological data provide similar results [9].

* Corresponding author E-mail: mhmmdshhndh@yahoo.com

While occupational exposure among workers at smelters or battery recycling plants remains an occasional problem, the greatest public health problem at the present time is the exposure of young children to decaying fragments of leaded paint. At higher blood levels, Pb^{++} disrupts the function of endothelial cells in the blood brain barrier. This may lead to hemorrhagic encephalopathy, characterized by seizures and coma. This disrupts long-term potentiation, which compromises the permanent retention of newly learned information [10].

The effects of lead are the same whether it enters the body through breathing or swallowing. The main target for lead toxicity is the nervous system, both in adults and children. Long-term exposure of adults to lead has resulted in decreased performance in some tests that measure functions of the nervous system. Lead exposure may also cause weakness in fingers, wrists or ankles. It also causes small increases in blood pressure, particularly in middle-aged and elders. Lead exposure may also cause anemia. At high levels of exposure, lead can severely damage the brain and kidneys in adults or children. Kidney tumors have developed in rats and mice that had been given large doses of some kind of lead compounds. Studies of lead workers suggest that long-term exposure to lead may be associated with increased mortality due to cerebrovascular disease. Population studies suggest that there is a significant association between bone-lead levels and elevated blood pressure. Blood lead levels (PbBs) also have been associated with small elevations in blood pressure. Between the two biomarkers, bone lead appears to be the better predictor [11].

Animal studies have shown that exercise improves learning and increases hippocampal neurogenesis [19]. Exercise also modulates synaptic function in the adult brain by increasing hippocampal BDNF, which regulates certain properties of synaptic transmission [12]. Exercise has been demonstrated to increase circulating levels of BDNF in humans [13]. The results suggest a positive correlation between physical fitness and cognitive function. It was also shown that acute workout leads to improvements in performing a hippocampal-based cognitive task, which may be related to a concomitant increase in circulating BDNF concentrations. Although chronic voluntary physical activity has been shown to enhance hippocampus BDNF expression in animals, the effects of forced exercise on a treadmill have not been reasonably investigated. It was shown that with an acute low-intensity exercise that is minimally stressful, hippocampus activation and BDNF expression can be achieved lending support to the idea that mild exercise could yield to greater benefits in hippocampus functions compared to the more strenuous forms.

Most studies in rodents were carried out using voluntary exercise such as free wheel running [14], but few investigated the effects of forced exercise [17]. The latter, much used by humans [15], probably involves a degree of stress [15], which is difficult to control. Only few authors have studied, animals submitted for similar periods of time to other forms of stress besides the one provided by the physical exercise.

However, while most studies that correlated physical activity with BDNF expression used the ‘‘voluntary wheel running’’ model [16], a major critical issue is that the exercise parameters, (i.e. intensity, duration, and frequency), are highly variable and dependent on the motor activity of the animal. To resolve this, in this study, we used a special treadmill running protocol in order to examine hippocampus activation and BDNF expression and present evidence that acute workouts differentially affect the time-course of BDNF induction in various regions of the rat hippocampus.

Material and Methods

Fifty-day aged, male, Wistar rats (256-290 g, n= 40) were maintained under standard laboratory conditions (12-h light/12-h dark cycles), in room temperature of 22±2 °C and food and water ad lb. Animals were acclimated to the treadmill for one week before the main exercise protocol began [30].

Rats were randomly assigned to 1) basic group (Base-B, n = 10), 2) control group (lead-L, n=10) 3), lead injection group (acute pre, n=10) and 4) treadmill exercise with lead injection group (acute post, n=10). The forth group’s training was like the third group’s, followed by an additional acute training (2 min acute workout). All rats were weighed on a daily basis during the exercise training phase.

Experimental procedures and exercise training

Animals were acclimatized to ambient rearing conditions for 4–5 days in group housing conditions (four rats per cage) and then habituated to run on a treadmill (KN-73, Natsume Ltd., Japan) for a total of seven sessions, over 8 weeks. The running speed and distance were gradually increased from 15 to 22 m/min and from 25 to 64 m/min respectively. Belt speed was 10 m/min, a walking rate for adult rats, and a speed that improved Morris maze performance in the adolescent rat [18]. At the end of the belt were stationary wire loops, which were electrified. A mild shock (0.75 mA, 500 ms duration, 0.5 Hz rate) was delivered through these loops to motivate the rats to continuously walk on the moving belt and thus avoid foot shock. The wire loops were activated during all exercise sessions, and an experimenter monitored all the treadmill sessions.

Table1: The scheme of the training for 8 weeks

Training Days	Number of weeks Speed & time of trainings	1	2	3	4	5	6	7	8
1	Treadmill speed (m/min)	15	16	17	18	19	20	21	22
	Time (minute)	25	30	35	40	45	50	55	60
2	Treadmill speed (m/min)	15	16	17	18	19	20	21	22
	Time (minute)	26	31	36	41	46	51	56	61
3	Treadmill speed (m/min)	15	16	17	18	19	20	21	22
	Time (minute)	27	32	37	42	47	52	57	62
4	Treadmill speed (m/min)	15	16	17	18	19	20	21	22
	Time (minute)	28	32	38	43	48	53	58	63
5	Treadmill speed (m/min)	15	16	17	18	19	20	21	22
	Time (minute)	29	34	39	44	49	54	59	64

Rats quickly learned to stay on the belt and avoid shock, except for one rat, which would not stay on the moving belt, and thus was quickly removed from the exercise group. The rats of L, and acute post groups ran 5 days a week, with primary time of 25, up to 64min at the end of the 8th week (Table 1). The acute post group, like the acute pre-group, had trainings followed by an additional acute training (2 min acute workout).

Animals in the acute workout groups were also conditioned to the treadmill over a 1-week period but only for 10 min at 0.8 km/h for 3 days/week. These animals exercised for 8 weeks, 5 days/week. Immediately before sacrifice, animals were made to run on the treadmill at 1.6 km/h until exhaustion, which was defined as the animal touching the electrified grid at the rear of the treadmill five times in 2 min [19].

The S groups received physiological solution, and L and LT groups got %2 lead acetate (20 mg/kg, i/p) 3 days a week, during all the 8 training weeks., all the rats were kept in their own cages until sacrifice. Rats were sacrificed by decapitation approximately 16 h after last exercises. After an intraperitoneal injection of 1% ketamine (30 mg/kg) and zalayzine hydrochloride (4 mg/kg) the rats were rapidly decapitated and the brains were quickly removed. The hippocampus region of brain was quickly dissected out. Transverse sections of hippocampus were prepared using a McIlwain tissue chopper. Then they were frozen with dry ice and cryopreserved at -80 C for inset hybridization and BDNF ELISA experiments. Blood samples were mixed with 100 mg/ml of EDTA to suppress coagulation and were cooled with ice for further analysis.

Analysis of BDNF concentration

BDNF protein was assessed using the ELISA kit

(Demeditec Diagnostics GmbH, Kiel, Germany) according to manufacturer's recommendations. The hippocampus was individually homogenized in lysis buffer containing (in mM): 137 NaCl, 20 Tris–HCl (pH 8.0), Igepal (1%), glycerol (10%), 1 PMSF, 0.5 sodium vanadate, 0.1 EDTA and 0.1 EGTA. Then it was centrifuged for 10 minutes at 14000 rpm and 4 °C. Supernatant was diluted in the sample buffer and was incubated on 96-well flat-bottom plates, previously coated with anti - BDNF monoclonal antibody. Standards, controls and samples were added. The plates were then incubated with Biotinated Secondary monoclonal antibody for 2 h and with Streptavidin horseradish peroxides - for 1 h. Then color reaction to substrate reagent (Tetraethyl Benzedrine) was quantified in a plate reader at 450 nm. The mean absorbance for each set of duplicate standards was controlled and sampled. The standard BDNF curve ranged from 0 to subtract the average zero standard optical density were calculated. Standard curve was plotted using Elisa 16 ng/ml.

Data analysis

Statistical analyses were conducted by two-tailed (Student's t-test)or one-way ANOVA followed by Tukey's test, when indicated. All data are presented as mean (±SEM).

Table 2: Hippocampus BDNF values in different groups at the 8th week

Groups	Number of rats	Mean nm/ml of protein	Std. Deviation	Std. Error	Minimum	Maximum
Base	8	1,8375	0,14772	0,05223	1,65	2,11
Lead	7	1,5400	0,21602	0,08165	1,24	1,87
Acute-pre test	6	1,8050	0,90447	0,36925	0,25	2,61
Acute-post test	7	2,0814	0,70674	0,26712	0,63	2,72

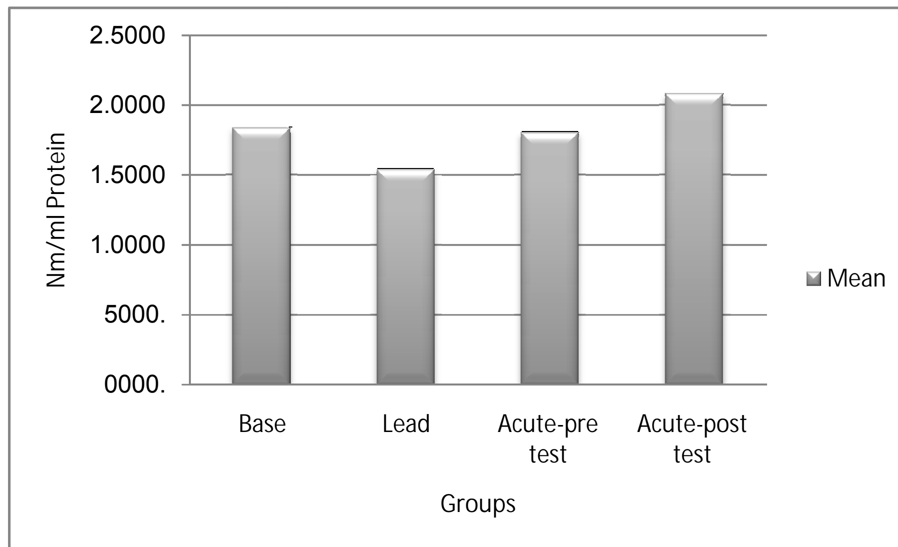

Figure 1: BDNF mean values in different groups

Results

Experiments were performed on 40 Wistar, male, rats. Available results obtained from isolated studies suggest that exercise alters BDNF levels and oxidative status. Results of our experiments indicated that at the end of the tests (the 8th week) the value of BDNF in L group (1,54 ± 0,21602 SD, n=7) reduced significantly in comparison with that of the base group (1, 8375 ± 0,14772 SD, n= 8, Table 2, Fig.1) (Table 2, Fig. 1). After trainings (see the scheme of training in Table 1) and at the end of the 8th week, the BDNF value of acute-pre and acute-post groups increased up to 1,8 (1,8050 ± 0,90447 SD, n=6) and 2,08 (2,0814 ± 0,70674,n=7) (Table 2,. Fig.1)respectively. The acute post group, like the acute pre group, had trainings followed by an additional acute training (2 min acute workout).

Discussion and Conclusion

It has been described that physical activity

increases the levels of some of the neurotrophins family members, especially BDNF, that modulates neuronal survival and plasticity [20], maturation and outgrowth in the developing brain, and exerts neuroprotective functions in the mature brain submitted to metabolic insults [21]. In addition, exercise induces BDNF mRNA in the hippocampus [22]. There is evidence indicating that physical activity may reduce age-induced cognitive decline and is recommended as a therapeutic strategy to prevent, or recover from, neurodegenerative disease [23]. Although the exact molecular mechanisms by which physical exercise affects brain function are unclear, it has been suggested that it might activate cellular and molecular pathways that contribute to neuroprotection.

The functional benefits of physical exercise on brain function have been studied in humans [24, and 40] and also in laboratory animals, especially rodents. Regular physical activity has been related to improvement of cognitive function in rats [25]. Physical exercise modulates hippocampal neurogenesis [26], reduces oxidative stress [27], increases brain-derived neurotrophic factor levels [28], and brain vascularization [29], and causes a variety of morphological changes.

Thus, the obtained data indicated the significant role of exercises in increasing BDNF levels. On the other hand the increased level of BDNF may act as a neuroprotector in recovering from numerous disturbances, such as reduction of the brain weight, memory loss and different degenerative processes. It may be concluded that BDNF increase in hippocampus may cause positive plastic changes and provide the prevention of the mentioned disturbance.

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