

The effects of chronic and acute physical and psychological Stress on Brain-Derived Neurotrophic Factor in Rats

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Received: 11 March 2018/ Accepted: 28 April 2018

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Abstract

Introduction: Different kinds of chronic stress can induce various effects on body systems including the brain. One of the factors related to brain function is brain derived neurotrophic factor (BDNF). So, the purpose of the present study was to evaluate the influence of physical stress as aerobic exercise/training and psychological stress on brain derived neurotrophic factor (BDNF) in Wistar rats.

Material & Methods: The study was semi experimental. 90 healthy male Wistar rats (weight 200 ± 40 gr) were randomly divided in to 6 groups of Exercise (EX), Emotional stress (ES), Physical stress (PS), exercise combined with emotional

(EXES), exercise combined with physical stress (EXPS) and control. Wistar rats were exposed to programs included one session (acute) and two weeks (chronic) aerobic training on treadmill with or without emotional and physical stress. Blood samples, for BDNF measurement, were taken 12 hours following the last session of treatment. Statistical tests of analysis of variance and follow up Bonferroni test were used for data analysis.

Results: After one session of the experiment, BDNF increased significantly in the EX group compared to other groups ($p < 0.05$). After two weeks of training, BDNF significantly decreased in the ES groups compared to other groups, while BDNF increased in EXES compared to ES ($P < 0.05$) group. One session of EX increased BDNF compared to non-EX groups, but following two weeks, chronic ES per se reduced BDNF compared to non-ES groups. But when ES combined with EX caused increasing of BDNF.

Conclusions: Present findings suggest that EX can probably prevent decreasing effect of ES on BDNF. However, future research should clarify the source of BDNF changes.

Keywords: Brain-Derived Neurotrophic Factor, Aerobic exercise, Emotional stress, Physical stress.

1. Introduction

Brain-derived neurotrophic factor (BDNF) is a division of neurotrophin family which is greatly expressed in mammalian brain (1) and some peripheral tissues including muscles (2), and adipose tissues (3). BDNF involve in central metabolic pathways (4), energy metabolism in peripheral organs (5), proliferation, differentiation, and survival of neurons, neurogenesis, synaptic plasticity, cognition function (6), body weight control, and feeding behavior (7). Several factors including gender, age and weight effect on BDNF levels in human (8).

Recent evidences have indicated the relationship between physical activity and BDNF level. A recent review study by Haung et al (2013)

reported that acute and chronic aerobic EX increase blood BDNF level (9). But according to majority of studies, reported in this review, the strength training was not effective on blood BDNF. Surprisingly, inverse relationship has been reported between habitual physical activity and physical fitness. So, there are many controversies related to the influence of EX or physical activity on blood BDNF. Recent findings suggest that blood BDNF is negatively related to some disease such as psychological job stress and uncontrolled neuron hyperactivity may suppress BDNF synthesis (10) but the cause of this suppression (peripheral, central or both) is not clear. Rodent studies have indicated significant relationship between blood and cortical BDNF levels (11, 12), suggesting that peripheral serum BDNF may help as a substitute for cortical measurements.

Although, the association between EX, physical activity and mental health (13) and cognitive impairments (14) have been proved by many studies, but sport participation may impose somatic, social and mental stress (15). However, fewer studies have conducted on physiological influences of different stresses (16) including exercise or sport.

Stress is defined as the conditions that are challenging emotionally and physiologically (17). The capacity to cope with stress is individual, but is accompanied by deregulation of active processes of adaptation, or allostasis, and cause allostatic load (17). Previous studies have indicated that chronic stress impairs muscular recovery of function and somatic sensations (18) and decrease muscular and neural adaptations (19, 20).

Sometimes EX is a stress by itself (21). Acute and chronic EX may be regarded as a stress for neuroendocrine system or play a dual role as a stress and a modifier of stress within the neuroendocrine system (22). Considering the relationship between physical activity or EX and mental stress (23), and potent influence of stress on BDNF (24) or different modulation of BDNF transcript by physical EX and stress (25), it is not clear yet that while EX is/is not accompanied by mental or physical stress, how it can influence on BDNF. Therefore, considering discrepancies in findings about influence of EX and ES on BDNF, we compared and studied the acute and chronic influence of combined and separate exercise, physical and emotional stress on serum BDNF in rats.

Material & Methods

Samples

90 Male wistar rats aged 3 months, weight 200 ± 40 were obtained from animal laboratory of Shiraz University of Medical Sciences. They were kept in constant situation including temperature (22 ± 1 ° C), light (from 7:00 am to 7:00 pm). All rats were given a standard rat diet and water were ad libitum throughout the study. Wistar rats were randomly divided to 6 equal acute and chronic treatment groups (every group included 8 rats): exercise (EX), emotional (ES), Physical stress (PS), exercise combined with emotional stress (EXES), exercise combined with physical stress (EXPS) and control.

This study was approved by local university ethic committee and was performed according to protection and ethical rules for animal studies in Shiraz University as well as the "Principles of laboratory animal care" (26) were followed.

Exercise program

Rats performed aerobic EX on treadmill for one session (acute) or two weeks (chronic). Before the main EX performance, all rats were placed on the treadmill for 10 min during the first week to reduce the novelty-induced stress. During the first day of the second week an incremental test was performed on the rodent treadmill to determine the physical EX intensity which was applicable in the training period. The indirect measurement of oxygen uptake (VO_2) peak was measured as recommended by Brooks and White (1978)(27). Each rat could run for about 23 min on the treadmill at a low-speed and then the speed was increased to 5 m/min every 3 minutes until the rats were exhausted (i.e., could not continue running). The time to exhaustion (in minutes) and workload (expressed by velocity in m/min) were recorded as indices of EX capacity, and for estimating VO_2 peak.

The EX intensity was controlled according to this index. From the second day, all rats performed aerobic EX 50 min/day 5day/week by the intensity of 60-75% of the maximum oxygen uptake. Each EX session initiated with a 10 min-long warm-up (gradual increasing of speed) followed by 30 min EX at intensity of 60-75% of the maximum oxygen

consumption. During the last 10 min of each session the speed reduced gradually for cool down.

The EX performed between 9:00 to 12:00 AM. Neither electrical shock nor physical prodding was used in EX sessions and by tapping on the back of animals they were encouraged to continue running in the case of refusal. Not being able to run was a criteria for exclusion of rats. The sedentary rats were kept in the similar running and shocking room on the still treadmill or stress box but they did not perform EX or receive any shock [adapted from Scopel et al., 2006 (28)].

Stress protocol

The rats were exposed to acute (one day) or chronic stress (2 weeks) mental or physical stress during 9 to 12 am. We used a 50 × 25 × 25 cm acrylic box which was divided into several rooms and rooms were equipped with electrical shock intermittently. In the electrical shock rooms, the physical stress group of rats received a 0.5 mA, 1-seconds foot shock every 30 seconds during 10 min , five times a week during one session per day for two weeks. No apparent tissue damage occurred in the footpads of shocked rats. The ES groups just could see the physical stress group but they did not receive any electrical shock.

The combined stress and EX groups performed running and received one kind of stresses at 9:00 to 12 am. Stressed was administered immediately after running on treadmill.

Sampling procedures

Regarding that blood BDNF reflect the brain tissue BDNF in rats (12), BDNF was measured through blood sampling. Blood samples(2 ml) were taken from ventral caudal artery of lightly etherized rats immediately after the first main treat treatment and 12 hours following the last session of treatment(after two weeks). The blood samples were centrifuged for 10 min (3500 rev) and were kept in -20 °C. BDNF levels were determined by enzyme linked immunosorbent assay (ELISA) using Rat BDNF ELISA kit(made in Germany) according to related recommendations.

Statistical analysis

Data were evaluated by one way analysis of variance (ANOVA), and in the case of significant findings followed by Bonferroni test. The results expressed as the mean \pm standard deviation. The significant level was set as $P < 0.05$. Statistical analysis was performed using the Statistical Package for social Science (SPSS) version 20.0.

Results

The BDNF descriptions (mean, SD, maximum and minimum) after the first session (acute response) and after 2 weeks (chronic) are presented in tables 1 and 2.

One way ANOVA test indicated a significant difference between study groups in BDNF following the one session of EX ($F=4.468$, $P=0.002$) (table 1). Bonferroni test indicated a significant difference between EX compared to ES ($P= 0.004$), EX compared to PS ($P= 0.023$), EX compared to control ($P=0.018$) groups. There was no significant difference in other paired group comparisons ($P > 0.05$). We found that one session of EX increased BDNF compared to non-EX groups.

Table 1. Between groups comparison of Plasma BDNF (pg/ml) after acute experiments.

groups	number	Mean \pm SD	F	P
EX*	12	14.76 \pm 1.61	4.468	0.002
ES	15	8.75 \pm 2.13		
PS	15	10.34 \pm 2.51		
EXPS	13	12.88 \pm 1.70		
EXES	11	12.04 \pm 2.18		
Control	15	10.00 \pm 2.82		

*significant difference ($P < 0.05$) compared to ES, PS and control

Comparison of BDNF between study groups following two weeks of study periods , using one way ANOVA test indicated a significant between group difference($F= 22.61$, $P=0.001$) (table 2). Paired group comparisons using Bonferroni follow up test indicated a significant difference in EX compared to ES ($P=0.001$), ES compared to PS ($P=0.003$), ES compared to EXES ($P=0.001$), ES compared to EXPS ($P=0.001$), ES compared to control ($P=0.002$) groups. We found that

ES per se reduced BDNF compared to non-ES groups. But when ES combined with EX, in EXES group, increased BDNF.

Table 2. Between groups comparison of Plasma BDNF(pg/ml) after chronic experiments.

groups	Number	Mean \pm SD	F	P
EX	10	13.37 \pm 1.09	22.63	0.001
ES*	13	7.39 \pm 1.55		
PS	14	11.54 \pm 1.32		
EXPS	12	15.03 \pm 0.86		
EXES	10	15.76 \pm 0.47		
Control	13	11.01 \pm 1.35		

*significant difference ($P < 0.05$) compared to EX, PS, EXPS and EXES

4. Discussion

There are different types of stress which can cause various effects. EX or physical activity is a kind of stress, which is recommended for reducing mental stress meanwhile athletes confront EX and mental stresses. So, evaluating the influence of different acute and chronic stresses, separately or in combination, on physiological responses, including BDNF is important which was studied in the present research. We found that one session of EX increased BDNF compared to non-EX groups, but following two weeks, chronic ES per se reduced BDNF compared to non-ES groups. But when ES combined with EX in EXES group caused increasing of BDNF.

Some other study findings (29, 30) confirmed our findings and indicated that acute aerobic EX increase BDNF. Although, the mode, type, intensity and duration of exercise were heterogeneous in these studies. But, Zoladz et al. (2008) reported no effect of one session of EX on BDNF (31). Regarding chronic EX, Shiffer et al. (2009) confirmed our findings and found that chronic aerobic EX did not significantly change BDNF level (32), while Some studies have indicated that chronic aerobic or resistance EX can increase BDNF (31,33-35).

Limbic-hypothalamo pituitary-adrenal axis and its related hormones, including corticotropin-releasing hormone, adrenocorticotrophic hormone and Glucocorticoids has an important role in adaptation to some stresses

including EX (37). Glucocorticoids can increase the release of excitatory amino acids (38) which can cause toxicity of hippocampus characterizing by neural atrophy and significant decrease of cell numbers (39) and may be related to BDNF changes. BDNF and its receptor TrkB play an important role during stress injury and elevation of BDNF mRNA and TrkB mRNA has been observed following acute injuries such as cerebral trauma (40). According to the most of studies chronic and repeated stress, decrease expression of BDNF. BDNF mRNA increased in pituitary glands if stress induced for 60 min and decreased following stress for 180 to 300 min (41). BDNF, directly or indirectly may contribute to stress protection and if the chronic neural damage occur (42), it can cause the reduction of BDNF. In the present study acute EX caused significant increase of BDNF, while two weeks of EX caused no significant influence on BDNF. Probably this period of time was not sufficient to induce any significant effect or some neural adaptations have occurred.

However, regarding emotional and physical stress we found different findings compared to the EX. We found that chronic ES caused significant reduction of BDNF, but while it was combined with the EX, caused increasing of BDNF. Two conclusions can be obtained by this finding: 1- chronic ES can cause degeneration of neural cells and 2- EX by different mechanisms from ES has a neural protective effect. Some previous studies have confirmed our findings and indicated that stressful conditions reduce BDNF expression (43, 24). In adult male rats, chronic application of foot shock caused decreased of BDNF (44), while acute foot shock increased BDNF in hippocampus of female but not male rats (45). Molteni et al. (2004) reported that EX reverse the reduction in BDNF in especial situations (46). Possible biological mechanism is that, EX may oppose the harmful effect of stress on neural plasticity and as a result BDNF production. Although, some studies have indicated that EX counteract down regulation of BDNF as a result of stress (47), but the exact related physiological mechanism is not clear yet. Kavushansky et al (2009) suggested that psychophysical stress (electric foot shock) differs from psychosocial (social defeat) stress in the expression of plasticity related genes (neural pathways) in the rat (48). It has been indicated that social stress causes down regulation of BDNF transcripts through

increasing the dimethylation levels of histone H3 lysine 27(H3K27me2) at the corresponding BDNF promoters (49). There are also some evidences that specific stresses may cause specific responses which may be related to activating different brain systems (50).

One limitation of this study was not measuring of hippocampus BDNF, and because the probable influence of other sources of BDNF in different stressful conditions is not clear, measurement of influences of different kinds of stresses on hippocampus as well as peripheral BDNF is recommended for future studies.

5. Conclusion

In conclusion , our findings suggest that in short time EX is the most important provoker stress for increasing BDNF, but in long time ES is the most important reducing stress for BDNF and when chronic EX combined with ES the response of BDNF is reverred and cause increasing of BDNF. So, EX probably could be a suitable treatment to enhance brain capability to deal with stressful conditions. However, since controlling of environmental stress in human subjects was almost impossible, this study was conducted on rats, and also regarding the mentioned limitations of this study, application of the present findings in human subjects needs more clarifications by future studies.

Conflict of interests: There was no conflict of interest among authors.

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