Iranian Journal of Basic Medical Sciences
Vol. 13, No. 3, Summer 2010, 146-153
Received: Dec 22, 2009; Accepted: Apr 25, 2010

IJBMS

Original article

The Pattern of Brain-Derived Neurotrophic Factor Gene Expression in the Hippocampus of Diabetic Rats

[1]Iraj Salehi, [2]Safar Farajnia, [3]Mustafa Mohammadi, *[4]Masoud Sabouri Ghannad

Abstract

Objective(s)
The aim of this study was to evaluate the effects of regular exercise in preventing diabetes complication in the hippocampus of streptozotocin (STZ)-induced diabetic rat.

Materials and Methods
A total of 48 male wistar rats were divided into four groups (control, control exercise, diabetic and diabetic exercise). Diabetes was induced by injection of single dose of STZ. Exercise was performed for one hr every day, over a period of 8 weeks. The antioxidant enzymes (SOD, GPX, CAT and GR) and oxidant indexes with brain-derived neurotrophic factor (BDNF) protein and its mRNA and apoptosis were measured in hippocampus of rats.

Results
A significant decrease in antioxidant enzymes activities and increased malondialdehyde (MDA) level were observed in diabetic rats (P= 0.004). In response to exercise, antioxidant enzymes activities increased (P= 0.004). In contrast, MDA level decreased in diabetic rats (P= 0.004). Induction of diabetes caused an increase of BDNF protein and its mRNA expression. In response to exercise, BDNF protein and its mRNA expression reduced in hippocampus of diabetic rats.

Conclusion
Diabetes induced oxidative stress and increased BDNF gene expression. Exercise ameliorated oxidative stress and decreased BDNF gene expression.

Keywords: BDNF, Hippocampus, Oxidative Stress, Exercise

1- Department of Physiology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
2- Biotechnology Research Center & Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
3- Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
4- Department of Microbiology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
*Corresponding author: Tel: +98-811-8276295-8; Fax: +98-811-8276299; email: sabouri39@yahoo.com

Introduction

Diabetes mellitus makes wide range of complications including the peripheral nervous disorders (1). Moreover, there are evidences that diabetes also threats the central nervous system (CNS) (2). It is clear that increased levels of reactive oxygen species (ROS) are engaged in pathogenesis of diabetes mellitus in both animals and humans. On this basis it may be inferred that oxidative stress has a role in the pathogenesis of diabetic neuropathy (3), so that the interaction of produced hydrogen peroxide with iron in the cells of CNS could be harmful. This is due to the generation of highly reactive hydroxyl radicals which results in severe damage to DNA and proteins (4). Because of the CNS high sensitivity to ROS, the maintenance of the normal redox seems vital in diverse types of neuron cells. In the recent years, attention has being drawn to potential role of brain-derived neurotrophic factor. BDNF is considered as one of the most essential neurotrophic factors in the brain which provides a critical mechanism in learning process, behaviors, locomotion, memory and a broad spectrum of stress responses (5). It has been proposed that BDNF regulates, promotes neurogenesis, neuroplasticity and cell survival in CNS (6). On the other hand, 4-methylcatechol, a stimulator for BDNF synthesis protects brain cells against neuropathy induced by diabetes in rats (7). Moreover, BDNF has been reported to stimulates the production of pro-survival Bcl-2 family members, antioxidant enzymes and proteins involved in calcium regulation which leads to prevention of cultured neurons death *in vitro* (8).

It has been reported that in a regular exercise, antioxidant defense systems are up regulated to restore the normal redox (9). It has became clear that regular exercise beneficially affects brain function as well, suggesting that it could play as a significant preventive and therapeutic action in brain and neurodegenerative disease (10, 11). The effect of exercise in the CNS is very complex and is not entirely understood but it is likely to be due to the action of neurogenesis via neurotrophic factors, increased capillarization and decreased oxidative damage (12). However, the exact mechanisms of up regulation of the BDNF expression were not similar in different brain regions, also the results of ample studies are the subject of conflicting reports. The main aim of this research was to evaluate the effects of diabetes on the hippocampus and also looking for the effects of regular exercise on BDNF and apoptosis in the hippocampus.

Materials and Methods

Animals

A total of 48 male Wistar rats (12-week old and initial weights of 235±27 g) were taken from laboratory animal house of Tabriz University of Medical Sciences located in the north western region of Iran. They were housed in an animal room at 22–24 ºC and given free access to tap water and rat chow. The animals were kept in 12:12 hr. light: dark round (light period 08.00–20.00 hr.). All the experimental procedures including rat care and handling were employed, which were in agreement with the guidelines provided by the Experimental Animal Laboratory and approved by the Animal Care Committee of Tabriz University of Medical Sciences.

Induction of diabetes

The intraperitoneal administration of 50 mg/kg of streptozotocin (STZ) (Sigma, St, Lois, Mo, USA) induced the diabetes. STZ was dissolved in prepared 0.05 M citrate buffer, pH 4.5, at once before injection. To induce a severe diabetic state, the rats were kept for 8 weeks before beginning the experiment. In both plasma and urine, glucose concentrations were controlled once every week. The criteria for detecting diabetic rats were as follows: minimum plasma glucose of over 250 mg/dl. As the control, age-matched, no treated (citrate buffer only) rats were used.

Training program

Rats were divided into four groups including control, control exercise, diabetic and diabetic exercise randomly. All the rats in the trained group swam in swimming pool (r= 50 cm, depth= 50 cm, water temperature 32±2 °C) and

performed weekly six exercise sessions of 60 min. duration for 8 weeks. Swimming time was 9-12 am. The control rats were also delivered to the experimental room and kept the same as the animals under experiment but not placing them in the swimming pool.

Preparation of hippocampus homogenates

The brain was rapidly cut after decapitation, and hippocampus was rapidly dissected on ice-cold dissection board. Tissues were homogenized, using a 2-ml glass/glass homogenizer in diverse buffers based on the assays to be performed. To extract RNA, tissue samples were homogenized in 1 ml of TRIZOL reagent per 50 to 100 mg of tissue. For the measurement of concentrations of BDNF protein, buffer A containing 137 mM NaCl, 20 mM Tris (pH 8.0), 1% NP40, 10% glycerol, 1 mM PMSF, 10 mg/ml aprotinin, 1 mg/ml leupeptin, 0.5 mM sodium vanadate was used. For oxidative stress, buffer B containing 1 mM EDTA, 50 mM potassium phosphate, (pH= 7.5) was used. The homogenates were centrifuged at 14000 g for 30 min. at 4 °C. Then the supernatant was collected for analyses. Protein concentrations were calculated by the procedure of Lowry *et al* (13), using BSA as the standard.

Lipid peroxidation and total glutathione measurement

Lipid peroxidation was analyzed by measuring thiobarbituric acid-reactive substances (TBARS) in homogenates, as previously described by Draper and Hadley (14). The glutathione content of the hippocampus was determined by the method of Griffith (15) in homogenates prepared.

Antioxidant enzymatic activities

Superoxide dismutase (SOD) and glutathione peroxides (GPX) activities were determined, using a RANSOD kit (Randox labs. Crulin, uk) and a RANSEL kit (Randox labs.) respectively. Catalase activity (CAT) was measured as previously described by Aebi (16). Glutathione reductase (GR) activity was measured as described by Carlberg and Mannervik with some modifications (17).

Measurement of BDNF contents

BDNF content in the hippucampus tissue was determined with BDNF E-max ELISA system kit (Cat. No. G7610, Promega, USA).

Semi quantification of BDNF mRNA expression

The reverse transcriptase polymerase chain reaction (RT-PCR) was applied to evaluate the BDNF and mRNA levels, using β-actin as internal control. Total RNA was extracted from the hippocampus tissue, using Trizol. Primers for the respective genes of rat BDNF and β-actin were used. RT-PCR was performed with a GeneAmp Thermostable rTth reverse transcriptase RNA PCR kit (Perkin-Elmer, Oak Brook, IL) in according to the manufacturer's instruction. The forward and reverse primers for detection of BDNF cDNA were: 5’GAATTCATGACCATCCTTTTCCTTACTA TG3’,5'AAGCTTTCTTCCCCTTTTAATGGT CAG3' respectively. The housekeeping β-actin forward and reverse primers were used as the control with the following sequences respectively:
5'CCCTAAGGCCAACCGTGAAAAGATG3',
5'GAACCGCTCATTGCCGATAGTGATG3'
In short, 500 ng of total RNA was reverse-transcribed with 0.75 mM downstream primer by rTth polymerase in the presence of Mn^{2+} for 15 min. at 60 °C. The synthesized cDNA was amplified by PCR for 15 min with both upstream and downstream primers. The thermal cycle profile used for amplification was 28 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min. A portion (10 μl) of the PCR products was resolved by PAGE and visualized by ethidium bromide staining. The density of the BDNF PCR products was analyzed by scion image software (Scion Corporation) and was reported as the ratio of the sample density to the density of the β-actin PCR products amplified from the same RNA sample.

Quantification of apoptosis

Cell death detection ELISA kit (1544675, Roche Molecular Biochemicals, Mannheim, Germany) was used to detect the cytosolic histone-associated DNA fragmentation

quantitatively, in according to manufacturer's instructions.

Data analysis

All the experiments were performed at least in duplicate. Data were expressed as mean±SD and were analyzed by a two-way ANOVA. To test for the two main effects (diabetes and exercise training) and for the interaction between them a standard computerized statistical program, SPSS version 13.0 for windows software (SPSS INC, Chicago, IL, USA) was used. When a significant *P*-value was obtained, the LSD Tukey *post-hoc* test was employed to determine the differences between groups. A level of $P< 0.05$ was selected to indicate statistical significance.

Results

The average weights of the diabetic and normal rats were 158±19.2 and 275±33.1 g, respectively, 8 weeks after the injection of STZ. General behavior and spontaneous activity were the same in both groups.

There was a significant increase in MDA, lipid peroxidation biomarker, hippocampus content in the diabetic group in comparison to the control group. Exercise induced significant decrease in MDA hippocampus content in the diabetic exercise group. Important changes were not seen in tGSH hippocampus in the control and diabetes groups in comparison with the trained groups (Table 1).

A notable decrease in SOD, GPX, CAT and GR enzyme activities in hippocampus of the diabetic group was observed in comparing with the control group. Exercise induced considerable increase in SOD, GPX and CAT activity in the diabetic exercise group, but did not show a major effect on GRD activity (Table 2). The results show a large increase in BDNF contents and BDNF gene expression in the hippocampus of the diabetic group in comparing to the control group (112.5% increase in gene expression). Moreover, exercise induced large decrease in BDNF contents and BDNF gene expression in the diabetic exercise group in comparison to the diabetic group (104% decrease in gene expression) (Table 3 and Figure1). There was a remarkable decrease of apoptosis in hippocampus of the diabetic group in comparison to the control group. Exercise induced significant decrease of apoptosis in the diabetic exercise group in comparison to the diabetic group, but was unsuccessful to show a meaningful main effect on apoptosis when comparing to the control group (Figure 1).

Table 1. Effects of diabetes and exercise on oxidative stress marker in the hippocampus of rats.

Group	tSOD (u/mg protein)	CAT ×10 (u/mg protein)	GPX (u/mg protein)	GR (u/mg protein)
Control	4.66±0.20	0.29±0.02	155.78±3.28	114.83±9.55
Control exercise	5.32±0.25	0.39±0.02	175.75±6.52	108.66±8.57
Diabetic	2.79±0.61*	0.23±0.01*	101.10±6.61*	93.50±8.59
Diabetic exercise	4.54±0.58#	0.30±0.03#	156.28±12.41#	85.83±22.26

Results are mean±SD of independent preparations. *Significant differences between the control and diabetes groups ($P< 0.001$). # Significant differences between the diabetic and diabetic trained groups ($P< 0.001$).

Table 2. Effects of diabetes and exercise on antioxidant enzymes in the hippocampus of rats.

Group	MDA (nmol/mg protein)	tGSH (nmol/mg protein)
Control	8.62±0.63	0.30±0.01
Control exercise	8.39±0.67	0.30±0.03
Diabetics	13.46±0.82*	0.28±0.01
Diabetics exercise	8.83±0.51#	0.28±0.01

Results are mean±SD of independent preparations. *Significant differences between the control and diabetes groups ($P< 0.001$). # Significant differences between the diabetic and diabetic trained groups ($P< 0.001$).

Table 3. Effects of diabetes and exercise on BDNF contents and BDNF gene expression in the hippocampus of rats.

Group	BDNF (pg/mg protein)	mRNA-BDNF (Control group%)
Control	156.83±21.68	57.75±4.99
Control exercise	167.08±9.98	85.38±8.98
Diabetic	209.61±15.86*	122.75±14.64*
Diabetic exercise	155.40±15.27#	62.78±7.10#

Results are mean±SD of independent preparations. *Significant differences between the control and diabetes groups ($P< 0.001$). # Significant differences between the diabetic and diabetic trained groups ($P< 0.001$).

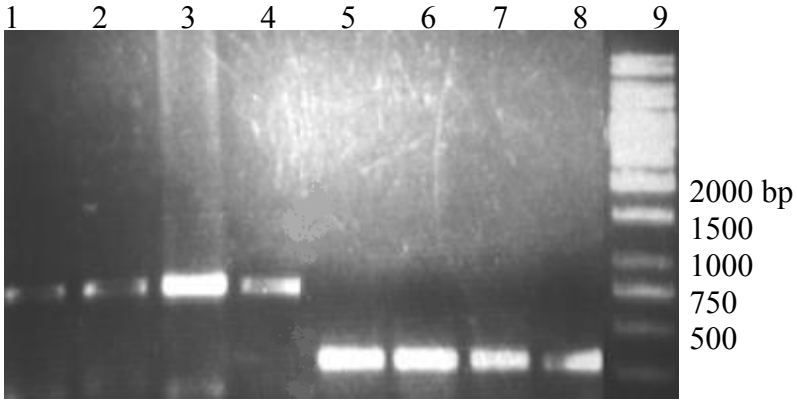

Figure 1. Effects of diabetes and exercise on BDNF gene expression in the hippocampus of rats. Electrophoresis of PCR product in the study groups. Respectively from the left to the right, BDNF (1-4), ß-actin (5-8), cDNA reactions in the control (1&5), control-exercised (2&6), diabetic (3&7) and diabetic exercised (4&8) groups. 1 Kb size marker is shown in line 9.

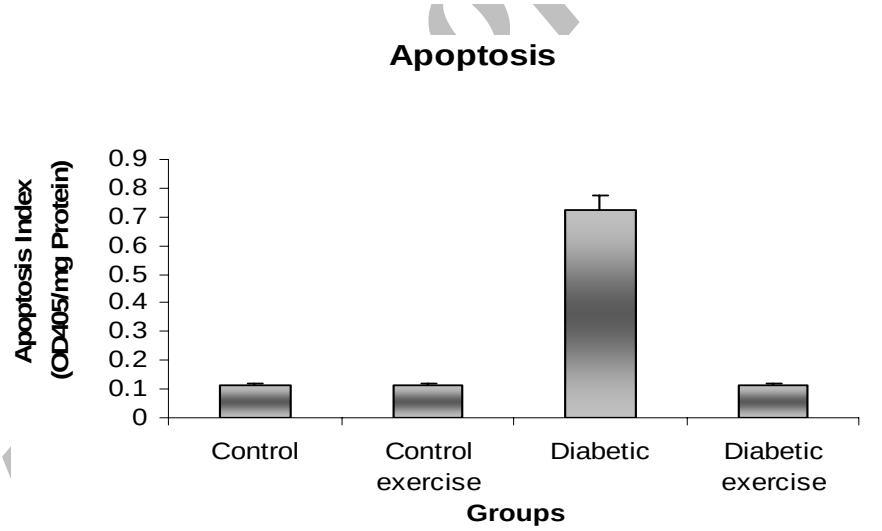

Figure 2. Effects of diabetes and exercise on apoptosis in the hippocampus of rats.

Discussion

The main aim of this research was to evaluate the effects of diabetes on the hippocampus and also looking for the pattern of BDNF gene expression. STZ-induced diabetes provides endogenous chronic oxidative stress (18). Numerous studies show that increased oxidative stress is a main contributor in the progress of diabetes and its complications (19-21). Our results demonstrates that diabetes was associated with increased hippocampus lipid peroxidation (Table 1); which is a well known marker of oxidative stress, inducing significant decrease in the antioxidant enzymes SOD, GPX, CAT and GR (Table 2). Induction of diabetes was more prone to up regulated expression of BDNF in hippocampus (Table 3, Figure 1). However, the effects of diabetes on the activities of antioxidant enzymes, MDA and GSH content in

hippocampus are the subject of conflicting reports and still a matter of debate (22). Brain oxidative damage is considered as a frequent etiopathologic mechanism of apoptosis and neurodegeneration. BDNF plays a pivotal role in this context. Our data showed that the expression and protein content of BDNF have been up regulated in the hippocampus of STZ-induced diabetic rats. BDNF has the potent cell survival–promoting effects, and it is thought to be used in the treatment of CNS diseases (23). There is considerable debate regarding the role of BDNF. Various results have reported different effects of this neurotrophin in the brain of diabetic rats. Some studies showed decreasing in expression of BDNF (24). In contrast, our findings are in agreement with some previous reports which showed an increasing in the expression of this neurotrophin in diabetes (25). It is not unusual for different or even contradictory conclusions to be reported in the literature for the same subject because of using the different models in various experiments. However, it is notable that based on our data hippocampus expression of BDNF was significantly increased in diabetic rats. This event could contribute to several aspects of different molecular interferences including an increasing in the production of ROS and inducing the apoptosis in hippocampus. Both in cell culture and *in vivo*, oxidative stress has been reported to induce apoptosis in neurons (26, 27). Decreased antioxidant defense seen in certain diseases could have further ROS-induced neuronal injury and apoptosis (28). A correlation was observed between oxidative stress and up regulation of BDNF expression (8). It has been shown that ROS stimulates the expression of BDNF, at least in cell culture, while antioxidant prevents the enhancement of BDNF (29). BDNF has been shown to protect the cells against glutamate-induced neurotoxicity *in vitro* (30), as well as ameliorating the severity of neuronal loss in neurodegenerative and ischemic models *in vivo* (31). Neurotrophic factors including BDNF have potential effects in protection of neuronal cells from traumatic and toxic brain injury (32). Increased expression of BDNF is in consistent with the above mentioned data already presented and confirms neuroprotective effect of BDNF against oxidative stress in hippocampus of STZ-treated rats.

Regarding the effect of exercise on the BDNF expression, our data indicated that swimming exercise modulated the oxidative stress and prevent increased expression of BDNF in hippocampus of the diabetic rats. Moreover, swimming exercise decreased apoptosis in hippocampus of diabetic rats.

It has shown that regular physical exercise modulates oxidative stress in diabetes (33). Only limited information is available at the current time concerning the effects of exercise on the oxidative stress status in brain and especially hippocampus in diabetes. Somani *et al*, 1996; Devi *et al*, 2004 reported that the effects of exercise on the activities of antioxidant enzymes were dependent on the brain regions, animal exercise protocol, strains or the detection assay techniques performed for the determination of enzymes activity (34, 35). Results of the present research is in consistent with some of the studies which indicate adaptive process (36) highlighting the therapeutic effect of regular exercise in diabetes, at least in part, which is due to oxidative stress-induced adaptation. Our study showed that the expression of BDNF and apoptosis in the hippocampus of diabetic exercise group did not have any significant difference in comparison to the control group. The major causative reason which can address this issue is that the modulated oxidative stress by exercise in this region of brain. Decline of oxidative stress remove the requirement of BDNF up regulation to prevent apoptosis in the hippocampus of exercised diabetic group. In consistent with the results obtained in this research, Tong *et al* (2001), reported that wheel running reduced the mRNAs expression of the apoptosis associated genes including Bcl-x, neuronal death protein and DP_5 (37). Some of the studies have shown the anti apoptotic and protective effects of BDNF in cultured neurons from excitotoxic damages *in vitro* and reduction of the severity of neuronal cell loss in degenerative and ischemic models in animals

(38). Moreover, some recent studies have confirmed that ProBDNF induces neuronal apoptosis via P75NTR (39). It seems that simultaneously increasing of BDNF along with apoptosis to be a defensive mechanism of neurons to escape from necrosis. The decline of the apoptosis by the swimming exercise may be related to the decreased oxidative damage. Therefore, there is no need for BDNF expression to prevent the destructive effects of oxidative stress.

Acknowledgment

This work was supported in part by a grant from Drug Applied Research Center of Tabriz University of Medical Sciences. We wish to thank Professor Hossein Babaei, for his support and advices.

References

1. Carsten RE, Whalen LR, Ishii DN. Impairment of spinal cord conduction velocity in diabetic rats. Diabetes 1989; 38:730-736.
2. Stewart R, Liolitsa D. Type 2 diabetes mellitus, cognitive impairment and dementia. Diabet Med 1999; 16:93-112.
3. Moorhouse CP, Halliwell B, Grootveld M, Gutteridge JM. Cobalt (II) ion as a promoter of hydroxyl radical and possible 'crypto-hydroxyl' radical formation under physiological conditions. Differential effects of hydroxyl radical scavengers. Biochim Biophys Acta 1985; 843:261-268.
4. Barde YA. What, if anything, is a neurotrophic factor? Trends Neurosci 1988; 11:343-346.
5. Berchtold NC, Chinn G, Chou M, Kesslak JP, Cotman CW. Exercise primes a molecular memory for brain-derived neurotrophic factor protein induction in the rat hippocampus. Neuroscience 2005; 133:853-581.
6. Phillips HS, Hains JM, Laramee GR, Rosenthal A,Winslow JW. Widespread expression of BDNF but not NT-3 by target areas of basal forebrain cholinergic neurons. Science 1990; 12:290-294.
7. Hanaoka Y, Ohi T, Furukawa S, Furukawa Y, Hayashi K, Matsukura S. The therapeutic effects of 4-methylcatechol, a stimulator of endogenous nerve growth factor synthesis, on experimental diabetic neuropathy in rats. J Neurol Sci 1994; 122:28–32.
8. Fleischer A, Ghadiri A, Dessauge F, Duhamel M, Rebollo MP, Alvarez-Franco F, *et al*. Modulating apoptosis as a target for effective therapy. Mol Immunol 2006; 43:1065-1079.
9. Radak Z, Chung HY, Goto S. Systemic adaptation to oxidative challenge induced by regular exercise. Free Radic Biol Med 2008; 44:153-159.
10. Mattson MP. Energy intake, meal frequency, and health: a neurobiological perspective. Annu Rev Nutr 2005; 25:237-260.
11. Mattson MP, Maudsley S, Martin B. A neural signaling triumvirate that influences ageing and age-related disease: insulin/IGF-1, BDNF and serotonin. Ageing Res Rev 2004; 3:445-464.
12. Nadai M, Yoshizumi H, Kuzuya T, Hasegawa T, Johno I, Kitazawa S. Effect of diabetes on disposition and renal handling of cefazolin in rats. Drug Metab Dispos 1990; 18:565–570.
13. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193:265–275.
14. Draper HH, Hadley M. Malondialdeyde determination as an index of lipid peroxidation. Methods Enzymol 1990; 186:421-431.
15. Griffith OW. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal Biochem 1980; 106:207–212.
16. Aebi H. Catalase *in vitro*. Methods Enzymol 1984; 105:121–126.
17. Carlberg I, Mannervik B. Glutathione reductase. Methods Enzymol 1985; 113:484–490.
18. Sano T, Umeda F, Hashimoto T, Nawata H, Utsumi H. Oxidative stress measurement by *in vivo* electron spins resonance spectroscopy in rats with streptozotocin-induced diabetes. Diabetologia 1988; 41:1355–1360.
19. Ceriello A. Oxidative stress and glycemic regulation. Metabolism 2000; 49:27–29.
20. Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: A new perspective on an old paradigm. Diabetes 1999; 48:1–9.
21. Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes 1991; 40:405–412.
22. Klepac N, Rudes Z, Klepac R. Effects of melatonin on plasma oxidative stress in rats with streptozotocin induced diabetes. Biomed Pharmacother 2006; 60:32-35.
23. Han BH, Holtzman DM. BDNF protects the neonatal brain from hypoxic-ischemic injury in vivo via the ERK pathway. J Neurosci 2000; 20:5775–5781.
24. Nitta A, Ohmiya M, Jin-nouchi T, Sometani A, Asami T, Kinukawa H, *et al*. Endogenous neurotrophin-3 is retrogradely transported in the rat sciatic nerve. Neuroscience 1999; 88:679–685.
25. Apfel SC. Neurotrophic factors in peripheral neuropathies: therapeutic implications. Brain Pathol 1999; 9:393-413.

26. Busciglio J, Yankner BA. Apoptosis and increased generation of reactive oxygen species in Down's syndrome neurons *in vitro*. Nature 1995; 21-28.
27. Park LC, Calingasan NY, Uchida K, Zhang H, Gibson GE. Metabolic impairment elicits brain cell type-selective changes in oxidative stress and cell death in culture. J Neurochem 2000; 74:114-124.
28. Facchinetti F, Dawson VL, Dawson TM. Free radicals as mediators of neuronal injury. Cellular Mol Neurobiol 1998; 18:667– 682.
29. Wang H, Yuan G, Prabhakar NR, Boswell M, Katz DM. Secretion of brain-derived neurotrophic factor from PC12 cells in response to oxidative stress requires autocrine dopamine signaling. J Neurochem 2006; 96:694-705.
30. Lindholm D, Dechant G, Heisenberg P, Thoenen H. Brain-derived neurotrophic factor is a survival factor for cultured rat cerebellar granule neurons and protects them against glutamate-induced neurotoxicity. Eur J Neurosci 1993; 5:1455–1464.
31. Beck T, Lindholm D, Castren E, Wree A. Brain derived neurotrophic factor protects against ischemic cell damage in rat hippocampus. J Cereb Blood Flow Metab 1994; 14:689–692.
32. Sendtner M, Holtmann B, Kolbeck R, Thoenen H, Barde YA. Brain-derived neurotrophic factor prevents the death of motoneurons in newborn rats after nerve section. Nature 1992; 360:757–759.
33. Radak Z, Sasvari M, Nyakas C, Kaneko T, Tahara S, Nakamoto H, *et al*. Single bout of exercise eliminates the immobilization-induced oxidative stress rat brain. Neurochem Int 2001; 39:33–38.
34. Somani SM, Husain K. Exercise training alters kinetics of antioxidant enzymes in rat tissues. Biochem Mol Biol Int 1996; 38:587–595.
38- Devi SA, Kiran TR. Regional responses in antioxidant system to exercise training and dietary vitamin E in aging rat brain. Neurobiol Aging 2004; 25:501–508.
35- Radak Z, Asano K, Inoue M, Kizaki T, Ohishi S, Suzuki K, *et al*. Acute bout of exercise does not alter the antioxidant enzyme status and lipid peroxidation in rat hippocampus and cerebellum. Pathophysiology 1995; 2:243–245.
36- Ozkaya YG, Agar A, Yargicoglu P, Hacioglu G, Bilmen-Sarikcioglu S, Ozen I, *et al*. The effect of exercise on brain antioxidant status of diabetic rats. Diabetes Metab 2002; 28:377–384.
37- Tong L, Shen H, Perreau VM, Balazs R, Cotman CW. Effects of exercise on gene-expression profile in the rat hippocampus. Neurobiol Dis 2001; 8:1046-1056.
38- Almli CR, Levy TJ, Han BH, Shah AR, Gidday JM, Holtzman DM. BDNF protects against spatial memory deficits following neonatal hypoxia-ischemia. Exp Neurol 2000; 166:99-114.
39- Henry K, Kenneth K, Ramee L, Saundrene W, Seema T, Ramiro D, *et al*. Hempstead. ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin. J Neurosci 2005; 25:5455–5463.