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Effects of Exercise on Stress-Induced Changes of Norepinephrine and Serotonin in Rat Hippocampus

Jing Wang, Xuewei Chen, Gaihong An, Na Zhang, and Qiang Ma

Department of Occupational Hygiene, Tianjin Institute of Health and Environmental Medicine Tianjin, People's Republic of China

Abstract

Exercise is beneficial to brain and can attenuate stress-induced hippocampal damages. However, the details involved monoamine neurotransmitter in exercise to counteract stress have not been well elucidated. The aim of this study was to examine exercise-induced responses of the norepinephrine (NE) and serotonin (5-HT) systems in counteracting stress-induced hippocampal damages. Rats were divided into exercise (four weeks of voluntary wheel running), stress (three weeks of restraint stress), exercise-stress (three weeks of stress following four weeks of exercise), and control groups. Levels of NE and 5-HT were detected with high-performance liquid chromatography (HPLC), mRNA expression was detected with real-time fluorescence quantitative reverse transcription polymerase reaction (FQ-RT-PCR) and proteins associated with 5-HT_{1A} receptors (5-HT_{1A}-R) and β_2-adrenergic receptors (β_2-AR) were analyzed by Western blotting. Serotonin levels were highest ($P < 0.01$) in the exercised group, lowest ($P < 0.05$) in the stressed rats, and were similar ($P = 0.065$) in stressed and exercise-stressed rats. NE levels were highest ($P < 0.01$) in the exercised group, and higher in the exercise-stressed than the stressed rats ($P < 0.01$). Serotonic receptor mRNA expression was highest ($P < 0.01$) in the exercised group, lowest in the stressed group. The 5-HT_{1A}-R protein expression changed in the same tendency as its mRNA levels. The β_2-AR mRNA was highest in exercised rats ($P < 0.05$), and its protein expression was higher in the exercised and exercise-stress rats than in the control and stress rats ($P < 0.05$). In conclusion, NE may represent endophenotypic features of exercise states. Serotonin levels may be more susceptible to stress and responsible for deleterious stress-induced effects. NE and 5-HT may both contribute to counteraction of stress-induced hippocampal damages of physical exercises.

Key Words: exercise, hippocampus, norepinephrine (NE), serotonin (5-HT), stress

Introduction

Long-term physical exercise and chronic psychological stress are well documented as two distinct sensory inputs to brain (5, 18). Exercise is beneficial and stress is harmful to neural functions and survival. This is particularly true for the hippocampus, a vital area of the brain for learning and memory function, as well as for behavioral regulation. Long-term regular voluntary physical exercise has been shown to enhance memory and cognition and hippocampal neuronal synaptic plasticity, promote detente gyrus (DG) neurogenesis, facilitate functional recovery following brain injury, and even ameliorate mental declines associated with aging (27). In contrast, chronic psychological stress has been reported to decrease learning and memory, induce dendritic atrophy and have a negative impact on brain plasticity (28).

Exercise is commonly believed to be a behavioral strategy for relieving stress, and it can reduce depression and anxiety in humans (3). Our previous animal study reported that eight weeks of voluntary wheel running exercise could attenuate three weeks of psychological stress that caused suppression of long-

Corresponding author: Dr. Qiang Ma, Department of Occupational Hygiene, Tianjin Institute of Health and Environmental Medicine, Tianjin 300170, People's Republic of China. Tel: +86-22-84655196, Fax: +86-22-84655017, E-mail: maqiangw@sina.com
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term potentiation (LTP) in rat hippocampal DG, thereby resulting in maintenance of normal plasma corticosterone levels (20).

The mechanisms for exercise to counteract stress have not been established. In this study, we tested the hypothesis that hippocampal functional changes involving norepinephrine (NE) and/or serotonin (5-HT) are necessary for exercise-induced protective effects. Evidence exists that NE and/or 5-HT are released within several brain areas as a result of exercise (24), and NE and 5-HT are two well-known classical neurotransmitters. Studies have demonstrated that NE and 5-HT can regulate synaptic plasticity, enhance neuronal survival, promote neural repair and improve mood (4, 11, 21). Therefore, we hypothesized that NE and 5-HT are key to exercise-involved protective effects. In addition, there is evidence that monoaminergic hypofunction is a treatable component of depression. Therefore, antidepressant medications have been designed to enhance NE and 5-HT neurotransmission (25). More specifically, 5-HT_{1A} and β_2-AR may need to be targeted for anti-depressant treatment because of their importance in regulating normal hippocampal function (13, 26). In this study, we evaluated changes in the mRNA and protein levels of these receptors as well as the NE and 5-HT levels following exercise or stress treatment, to explore mechanisms in counteracting stress.

Materials and Methods

Subjects

Twenty-eight male Wistar rats, average weight of 200 g, were obtained from the Animal Center in the Academy of Military Medical Science (Beijing, China). Rats were housed singly in standard stainless cages with food and water available *ad libitum*, and were kept in a temperature and humidity-controlled room on a 12-h (06:00-18:00) light/dark cycle. Rats were randomly divided into four groups of seven animals each: controls (control group); voluntary physical exercise (exercise group); restraint stress (stress group); restraint stress following four weeks of exercise (exercise-stress group).

Models

Voluntary wheel running was used as the physical exercise model. Exercise rats were housed singly in cages equipped with free access (24 h) to 26-cm diameter and 8-cm width running wheels for 4 weeks. Physical activity on the running wheels was voluntary, and the distances run per 24 h were approximately 2 km for each rat (12).

Restraint stress was used as the psychological stress model according to a previous report (17). Rats were individually immobilized for 6 h each day, starting at 09:00, in 15-19-cm long by 5-cm diameter iron restraint cylinders, over a three-week period. The exercise-stressed rats were permitted voluntary wheel running for four weeks, followed by 21 daily sessions of restraint stress that began two days after cessation of voluntary wheel running. Controls were sedentary, and singly housed without disturbances.

Tissue Preparation

All rats were killed at 09:00, two days after their last stress or exercise paradigm. Animals were rapidly decapitated, and their brains were quickly removed and dissected over ice to obtain bilateral hippocampi. All dissected hippocampi were quick-frozen and then stored in -196°C liquid nitrogen for later analysis.

Neurotransmitter Analysis

Hippocampal samples were analyzed using established methods for monoamine neurotransmitters, including NE and 5-HT, by high-performance liquid chromatography using electrochemical detection (HPLC-ECD) at -0.7 V (30). Briefly, samples were sonicated in 1.0 to 1.5 ml 0.1 N $HClO_4$ and 40 µl 3,4-Dihydroxy-benzylamine (DHBA) as the internal standard, and centrifuged at 14,000 rpm for 15 min. Supernatants (20 µl) were injected onto a C18, 4.5 mm × 250 mm, 10 µm chromatography column (Shimadzu, Nakagyo-ku Japan) in a mobile phase containing KH_2PO_4 (100 mM), OSA (1 mM), EDTA. Na_2 (0.5 mM), methanol (11% v/v) and pure water. Sample amounts were calculated by comparing the relative peak areas of sample peaks to external standards. NE and 5-HT were measured in a single chromatogram. Concentrations are expressed as milligram for NE, or nanogram for 5-HT, per gram of sample tissue wet weight. Results are expressed as mean ± standard deviation (SD).

Analysis of mRNA

Trizol was used to extract hippocampus neuronal total RNA. For each specimen, the absorbance at a wavelength of 260 nm was determined and the density of RNA was calculated as: Density (g/l) = OD260 × dilution multiple × 40/1,000. Total RNA (1 µl) was reverse-transcribed and amplified with FQ-RT-PCR (Light Cycle, Roche Diagnostics, Mannheim, Germany). The reverse transcription reaction system used was as follows: 5 × buffer 2 µl, Prim RT Enzyme 0.5 µl, Oligo dT Primer 0.5 µl, Random 6 mers 0.5 µl, RNase free dH_2O 5.5 µl, and RNA template 1 µl. The bulk volume was 10 µl. The buffer included dNTP

mixture and Mg^{2+}. The conditions of this reaction were as follows: 25°C for 10 min, 37°C for 1 h, 85°C for 5 min. The PCR reaction system was cDNA (1 µl) mixed with 2 × SYBR Premix Ex Taq 10 µl, forward primer (10 µM) 0.2 µl, reverse primer (10 µM) 0.2 µl, and dH_2O 8.6 µl. The total volume is 20 µl. SYBR Premix Ex Taq includes TakaRa Ex Taq HS, dNTP Mixture, Mg^{2+}, and SYBR Green. Samples were incubated at 94°C for 2 min followed by 40 cycles. Each cycle consisted of 95°C for 15 s, 60°C for 40 s followed by reading of fluorescence absorption.

With reference to the National Center for Biotechnology Information (NCBI) Genbank, the primers and probes were designed by TaKaRa Biotechnology Co., Ltd. (Dalian, China). Primers sequences were: 5-HT_{1A}-R Forward: 5'-CTGCCCATGGCTGCTC TGTA-3'; Reverse: 5'-CATCCAGGGCGATAAACA GGTC-3'; β_2-AR Forward: 5'-TCTGATGGTGTGGA TTGTGTC-3'; Reverse: 5'-ACGTCTTGAGGGCTT TGTGCT-3'. mRNA levels were evaluated as a relative unit determined by $2^{-\Delta\Delta Ct}$ (19).

Protein Analysis

Hippocampal samples were homogenized in a buffer containing 20 mM Tris-HCl (pH 7.0), 1 mM EDTA, 1% Triton-100, and the protease inhibitor, 0.5% phenylmethylsulfonyl fluoride (PMSF). The protein concentration of each homogenate was determined with a bicinchoninic acid (BCA) protein quantification kit according to the instructions. Proteins were heated to 100°C in a SDS-PAGE sample buffer (50 mM Tris-HCl, pH 6.8, 100 mM dithiotreitol (DTT), 2% SDS, 10% glycerol and 0.1% bromophenol blue, and loaded onto 10% polyacrylamide gels. After electrophoresis, samples were transferred to polyvinylidene difluoride (PVDF) membranes. Membranes were blocked in a blocking solution of Tris-buffered saline (TBS) containing 0.1%Tween 20 (TBST), 5% nonfat milk for 1 h. Membranes were incubated overnight at 4°C with anti-5-TH_{1A} and anti-β_2 AR antibodies (Santa Cruz Biotechnology, Santa Cruz, USA), diluted with 1/500 in TBST and 1% BSA. After three washes in TBST for 10 min, membranes were incubated with a secondary alkaline phosphatase antibody diluted 1/5,000 in TBST and 1% BSA, and the bound antibody was visualized by chemiluminescence. Differences in protein gel loading and blotting were assessed by re-incubating the membranes with an anti-GAPDH antibody. Incubation and washing conditions for the anti-GAPDH antibody were identical to those for the antibody above. The molecular mass of the band labeled with each antibody was scanned in a gel imaging half-quantitative analysis system.

Statistical Analysis

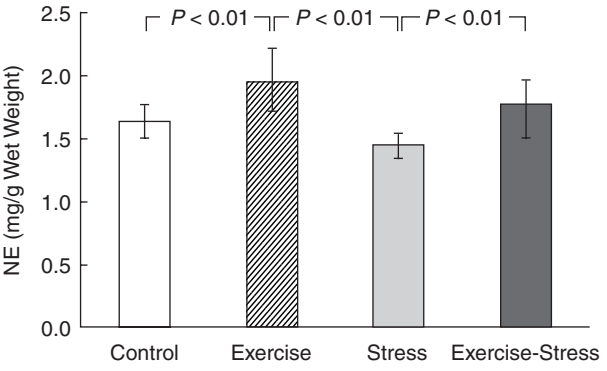

Fig. 1. Hippocampal NE levels in exercised, stressed and exercise-stressed rats. Significant differences ($P < 0.01$) were evident in comparisons between the exercise and control, the exercise and stress, and the stress and exercise-stress groups.

All values are presented as mean ± SD. Statistical analyses were carried out with one- or two-factor ANOVA using PASW statistics 18.0 for Windows.

Tukey's honestly significant difference (HSD) tests were used for multiple *post hoc* comparisons. Statistical significance was accepted at $P < 0.05$.

Results

Influence of Exercise and Stress on the NE Levels in Rat Hippocampus

The four weeks of wheel running exercise, three weeks of restraint stress, and the combination of four weeks of pre-exercise and three weeks of stress led to some changes in hippocampal NE levels in the experimental rats (Fig. 1). NE levels were significantly ($P < 0.01$) increased in exercised animals compared to the controls. Likewise, NE levels were also significantly ($P < 0.01$) higher in the exercise group (1.96 ± 0.25 mg/g), compared to the stress group (1.45 ± 0.09 mg/g). It is noted that there was no difference ($P = 0.081$) in the NE levels between the stress and control groups (1.64 ± 0.13 mg/g). However, the levels of NE were significantly higher ($P < 0.01$) in exercise-stressed rats (1.78 ± 0.26 mg/g) compared to the stressed rats ($F = 8.237$, $df1 = 3$, $df2 = 24$, $P < 0.01$). The value of F and degree of freedom (df) show that the significant changes was seen in statistical ANOVA analysis, on the bases of it, multiple *post hoc* comparisons were used.

Influence of Exercise and Stress on the 5-HT Levels in Rat Hippocampus

The four-week exercise, three-week stress and the combination of four weeks of pre-exercise and

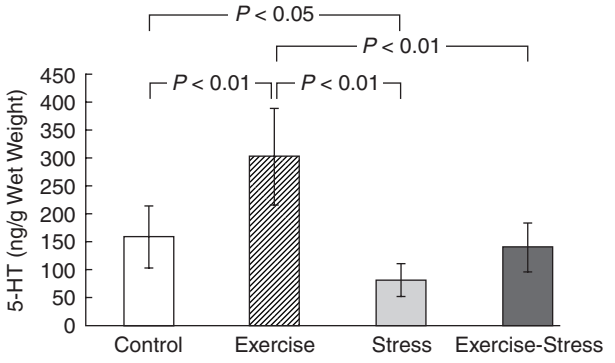

Fig. 2. Hippocampal 5-HT levels in exercised, stressed and exercise-stressed rats. Significant differences ($P < 0.01$) were evident in comparisons between the exercise and control, the exercise and stress, and the exercise and exercise-stress groups. There were significant differences ($P < 0.05$) between the stress and control groups.

three weeks of stress led to changes in hippocampal 5-HT levels in the experimental rats (Fig. 2). The 5-HT levels were significantly ($P < 0.01$) increased in exercised (303.88 ± 85.38 ng/g) animals compared to the other 3 groups. In contrast, 5-HT levels in stressed rats (81.54 ± 29.41 ng/g) were decreased ($P < 0.01$) compared to controls (158.87 ± 56.05 ng/g). However, there were no differences ($P = 0.065$) between the exercise-stressed (140.87 ± 43.27 ng/g) rats. There were also significant differences ($P < 0.01$) between exercise and the exercise-stressed rats. F = 18.940, df1 = 3, df2 = 24 ($P < 0.01$). The value of F and df show that the significant changes was seen in statistical ANOVA analysis, on the bases of it, multiple *post hoc* comparisons were used.

Influence of Exercise and Stress on the β_2-AR mRNA Levels in Rat Hippocampus

Compared to the other groups, hippocampal β_2-AR mRNA levels were significantly ($P < 0.05$) increased in the exercise group (2.58 ± 0.42 times) (Fig. 3). No significant differences were observed between stress and exercise-stress groups (1.38 ± 0.62/1.48 ± 0.55 times versus the controls. F = 11.683, df1 = 3, df2 = 24, $P < 0.01$). The value of F and df show that the significant changes was seen in statistical ANOVA analysis, on the bases of it, multiple *post hoc* comparisons were used.

Influence of Exercise and Stress on the 5-HT$_{1A}$-R mRNA Levels in Rat Hippocampus

Exercise and stress led to some changes in the mRNA levels in the experimental rats. Compared to controls (1.04 ± 0.27 times), hippocampal 5-HT$_{1A}$-R

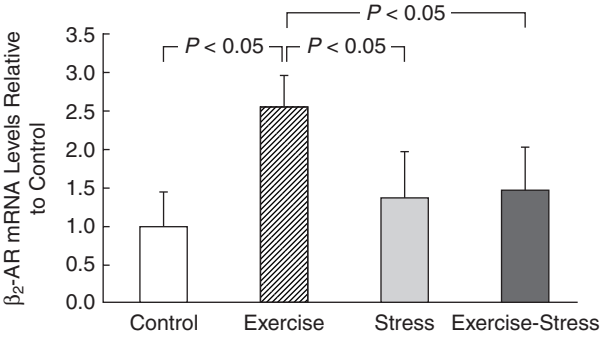

Fig. 3. Hippocampal β_2-AR mRNA levels in exercised, stressed and exercise-stressed rats. Significant differences ($P < 0.05$) existed between the exercise and other groups.

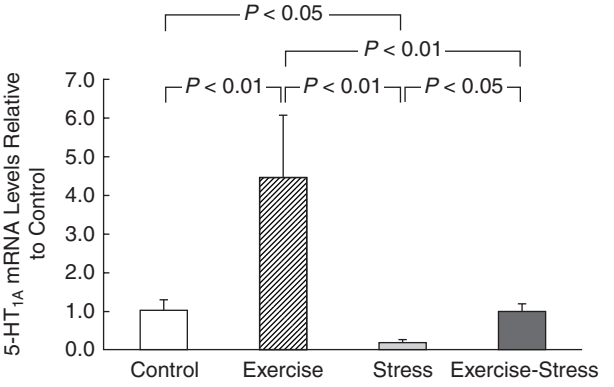

Fig. 4. Hippocampal 5-HT$_{1A}$-R mRNA levels in exercised, stressed and exercise-stressed rats. Significant differences ($P < 0.01$) existed between the exercise and other groups.

mRNA levels were significantly ($P < 0.01$) increased in the exercise group (4.49 ± 1.59 times) and decreased in the stress group (0.22 ± 0.55 times) (Fig. 4). Although mRNA levels in the exercise-stress group (1.00 ± 0.18 times) were increased ($P < 0.01$) compared to the stress group, they were still lower ($P < 0.01$) than the exercise group (F = 46.371, df1 = 3, df2 = 24, $P < 0.01$). The value of F and df show that the significant changes was seen in statistical ANOVA analysis, on the bases of it, multiple *post hoc* comparisons were used.

Influence of Exercise and Stress on β_2-AR Protein Expression in Rat Hippocampus

Compared with the control group (1.00 ± 0.46 times), no change was evident in the stress group (1.33 ± 0.58 times), but there were increases in the exercise (2.57 ± 0.42 times) and exercise-stress groups (2.41 ± 0.33 times, $P < 0.01$). Also, the same tendency was evident in the exercise and exercise-stress groups compared with the stress group (Fig. 5).

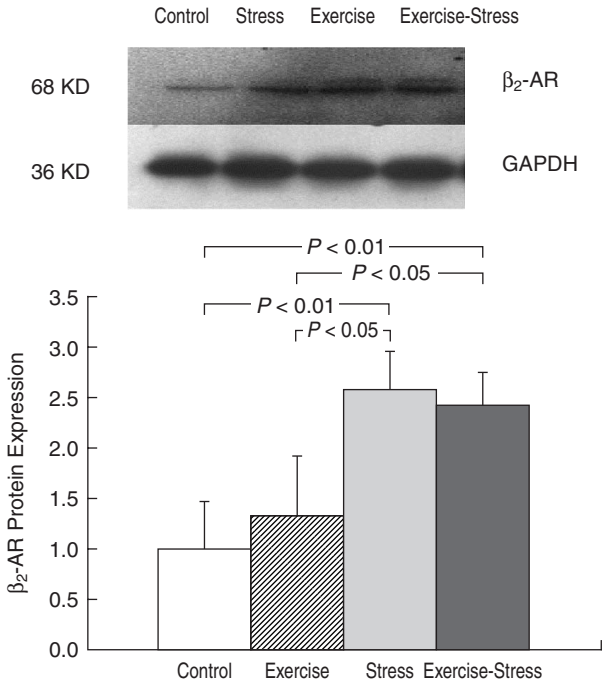

Fig. 5. Effects of chronic psychological stress and physical exercise on hippocampal β_2-AR protein expression levels of rats. Compared with the controls, no changes were evident in the stress group, but significant increases were apparent in the exercise and exercise-stress groups ($P < 0.01$). Also, similar results were apparent in the exercise and exercise-stress groups, compared with the stress group ($P < 0.05$).

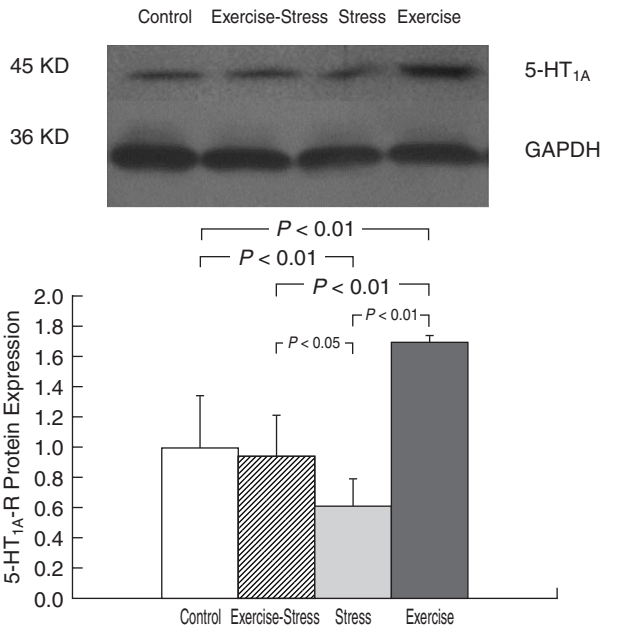

Fig. 6. Effects of chronic psychological stress and physical exercise on hippocampal 5-HT_{1A}-R protein expression in rats. Compared with the controls, no changes were evident in the exercise-stress group. Compared with the other 3 groups, significantly increases and decreases were apparent in the exercise and stress groups, respectively ($P < 0.05$).

(F = 19.394, df1 = 3, df2 = 24, $P < 0.01$). The value of F and df show that the significant changes was seen in statistical ANOVA analysis, on the bases of it, multiple *post hoc* comparisons were used.

Influence of Exercise and Stress on 5-HT_{1A}-R Protein Expression in Rat Hhippocampus

Changes in 5-HT_{1A}-R protein expression paralleled its mRNA levels. 5-HT_{1A}-R levels were increased in the exercise group (1.69 ± 0.04 times) compared with the other 3 groups, whereas levels were lowest in the stress group (0.61 ± 0.18 times). The protein expression for the exercise-stress group (0.93 ± 0.27 times) was higher than the stress group, but lower than the exercise group and similar to the controls (1.00 ± 0.33 times, Fig. 6). (F = 38.367, df1 = 3, df2 = 37, $P < 0.01$). The value of F and df show that the significant changes was seen in statistical ANOVA analysis, on the bases of it, multiple *post hoc* comparisons were used.

Discussion

Dentate granule cells in the brain send mossy fiber terminals to the CA3 and the hilus, which receives extrinsic noradrenergic and serotonergic afferents from the locus coeruleus (LC) and median dorsal raphe (MRN) areas, respectively. The NE and 5-HT systems in the hippocampus, which mediate neural signaling pathways, may play important roles in exercise/stress interactions (20). The 5-HT_{1A}-R subtype is the most abundant 5-HT receptor in the hippocampus, where a significant proportion is postsynaptic. The pathway connecting the MRN to the hippocampus promotes resistance to CS. 5-HT_{1A}-R receptors are the main target of the MRN-hippocampal pathway (17). Likewise, the β_2-AR subtype is present in high concentrations within the hippocampus and is located postsynaptically. The β_2-AR subtype is reported to be associated with downstream effects on brain-derived neurotrophic factor (BDNF) expression, which is an important neurotrophic factor for brain function and neuronal survival (17).

Our results show that significant changes occurred in the hippocampal NE and 5-HT levels and their receptor subtypes in the exercised rats, whereas only significant changes occurred in the 5-HT levels and associated receptor subtype responses in stressed rats. Additionally, NE levels increased significantly in exercise-stressed rats compared to stressed rats that were not exercised. On the other hand, the 5-HT

levels did not differ between the two groups, which were all significantly lower than the exercised rats. Notably, there were no obvious differences in the β_2-AR mRNA expression levels in the stress, exercise-stress and control groups. On the other hand, 5-HT_{1A}-R mRNA was expressed in higher levels in the exercise-stress than in the stress group, although the expression of this mRNA in both groups was significantly lower than the exercise groups. Consequently, it appears that both the hippocampal NE and 5-HT systems responded to exercise. Exercise can increase both the neurotransmitter and receptor subtype mRNA levels of NE and 5-HT, and counteract stress-induced 5-HT_{1A}-R mRNA decreases, while having no effects on decreases in stress-induced 5-HT levels. More research is warranted to establish the sensitivity of hippocampal 5-HT_{1A}-R to mild stress and a deficiency in BDNF. Since the decrease of 5-HT_{1A} autoreceptor density/function is associated to more severe depressive symptoms, therefore, effect of stress seems more important on the 5-HT_{1A}-R than on the 5-HT levels. Conversely, Bambico *et al*. reported that in CUS, 5-HT_{1A} autoreceptor desensitization is paralleled by a dramatic decrease in 5-HT neuronal firing activity (2). This desensitization can, therefore, be inferred as a compensatory response to a primary decrease in 5-HT electrical activity, which may in turn be directly induced by CUS (14). Otherwise, the 5-HT system appears to be susceptible to stress because stress can decrease both the 5-HT and 5-HT_{1A}-R mRNA levels, and has no effects on the NE system.

Central monoaminergic responses to physical exercise and stress are known. Previous studies have shown similar results regarding changes in NE and 5-HT levels induced by stress. Duman and Fontenot have shown that chronic stress can cause a pronounced decrease in hippocampal 5-HT levels as well as in 5-HT signaling (7, 10). Periods of stress lasting four weeks resulted in hippocampal NE changes that were initially increased, and then decreased (9). Central NE activities paradoxically have either anxiogenic or anxiolytic effects depending on whether the time course of the stress is acute or chronic, or whether the stress is predictable or unpredictable, and which underlying brain regions are affected (14). However, in our studies, NE levels appeared unchanged under chronic stress (CS). We believe that the duration of stress may be the key factor controlling stress-induced NE changes. There is also evidence that 5-HT_{1A}-R is down-regulated in CS (17). No evidence exists about hippocampal β_2-AR mRNA changes in CS. On the other hand, studies involving exercise-induced increases of NE and 5-HT activities support our results. Waters and Dishman reported that chronically exercised animals showed increased levels of NE and 5-HT in several brain areas, including the hippocampus, as compared to sedentary controls (6, 29). No evidence exists regarding hippocampal β_2-AR and 5-HT_{1A}-R mRNA changes under exercise.

Many studies have shown that physical exercise acts to counteract the detrimental effects of brain insults such as stress and aging. Studies on humans have revealed that physical activity is associated with lower risks of cognitive impairment, Alzheimer's disease (AD) and dementia in general (23). Studies on animals have also reported that exercise can counteract stress-induced decreases in BDNF and LTP expression to protect neurons against stress-induced neuronal degeneration (1, 20). Our results are consistent with these reports showing that exercise can counteract stress-induced damages to the central neuronal system. NE and/or 5-HT abnormalities contribute to the pathogenesis of AD, Huntington's disease, depression and other diseases. For example, lower levels of central 5-HT are associated with depression caused by chronic social stress. Studies have indicated that physical exercise is an effective intervention for improving central monoaminergic function and mood, as well as to counteract stress-induced neural disorders (5, 13, 15, 21).

Various studies disagree as to whether acute stress decreases or increases hippocampal 5-HT levels, but the difference of 5-HT levels appears to be a function of synthesis of active 5-HT and/or metabolism (8). However, there is not disagreement that CS decreases hippocampal 5-HT levels in response to chronic exposure to high levels of stress-induced glucocorticoids. Brain glucocorticoid receptors are mainly located in the hippocampus (15). It was reported that CS resulted in persistent deficits in presynaptic mechanisms that control 5-HT activity in the dorsal raphe (DR) nucleus, but impairments are not observed after (acute restraint stress, AcRes) (2). CS induced a significant decline in DR 5-HT single-spike firing. On the other hand, AcRes led to slight, non-significant increases in DR 5-HT single-spike firing activity. Furthermore, a significant increase in the percentage of burst firing neurons in comparison to controls was observed in the AcRes but not in the CS group. This burst activity in AcRes could be related to rapid increases of monoamine release in response to acute stress (2). Likewise, studies on exercise-induced fatigue revealed that endurance exercise can increase synthesis of 5-HT in the brain. The mechanism for this is a change from albumin-bound tryptophan to free tryptophan in peripheral blood, which can occupy a carrier for transport over the blood-brain barrier, and the decline of branched-chain amino acids (16). In spite of the lack of clarity and/or controversy, it is conceivable that final results following monoaminergic changes are related to alterations in BDNF levels, which may be the down-

stream targets of the exercise-induced changes. Chronic physical exercise can increase levels of BDNF and other growth factors to stimulate neurogenesis, to increase resistance to brain insult and to improve learning and mental performance (5). On the other hand, chronic stressors cause pronounced decreases in BDNF levels in the hippocampus and other brain regions. This has adverse effects on the brain, such as impaired synaptic plasticity and neurogenesis, and increased vulnerability of neurons to oxidative, metabolic and excitotoxic injury (7, 22).

A functional NE system meditated by β-AR is necessary for increased transcription of BDNF due to exercise. An intact NE system (but not the 5-HT system) may be crucial for exercise-induced enhancement of hippocampal BDNF mRNA expression (11). NE-activation may be a key pathway for exercise-induced benefitial effects on the brain. Although there is controversy about the exact role 5-HT plays in stress-induced brain damages and adverse emotional states such as depression and anxiety, Deakin and Graeff have suggested that the 5-HT pathway that is initiated in the MRN and innervates the hippocampus increases resistance or tolerance to chronic and unavoidable stress. They explained that the MRN-hippocampus 5-HT system attenuates stress by facilitation of hippocampal 5-HT_{1A}-R-mediated neurotransmission (16). Therefore, in agreement with Deakin and Graeff's theory and our results, CS down-regulates hippocampal 5-HT_{1A}-R-mediated 5-HT transmission. Above all, chronic wheel running activity in our study may partly increase hippocampal β_2-AR-R-mediated NE transmission, as well as 5-HT_{1A}-R-mediated 5-HT transmission, to counteract stress-induced adverse effects. It appears that 5-HT and NE reactions occurred independently and co-operatively in exercise and/or stress-induced groups, but little is known of these dynamics.

Both NE and 5-HT may play, important roles in mediating the exercise-induced positive effects. Norepinephrine may represent endophenotypic features of exercise states. Serotonin may be more susceptible to stress and plays critical role in stress-induced negative effects. NE and 5-HT may both act in the effects of exercise counter-acting stress-induced hippocampal damages.

Acknowledgments

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