Voluntary Wheel Running Exercise Ameliorates the Effect of Maternal Restraint Stress Induced the Increase in the Level of β-TrCP in the Hippocampus of the Rat Offspring

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Abstract

Introduction: The developing hippocampus is refined by the external environment, including maternal restraint stress. The enhancement of glucocorticoids (GCs) during stress causes a plethora of the effects to the pup hippocampus, where is high plasticity area. According to stress hormone elevates glutamate concentration in the brain, glutamate receptor is the key regulator of the developmental plasticity. Overstimulation of hippocampal neurons by glutamate causes the vulnerability of cognitive impairment and psychiatric disorders later in life. Our previous study showed that maternal stress induced an increase in the level of β-Transducin repeats Containing Proteins (β-TrCP), an E3 ubiquitin ligase. That was contrary to the depletion of the scaffolding proteins of NMDA receptor, Postsynatic density (PSD)-95 and Spine Associated Rap Guanylate kinase Activating Protein (SPAR) in the hippocampus of the rat offspring. Increasing evidence of physical exercise has rendered the positively modification at the cellular and molecular level in neurons. We thus examine the potential effect of voluntary wheel running on the level of β-TrCP.

Methods: The mother rats were divided into 2 groups, the non-stress control group and the restraint stress group. Voluntary wheel running exercise was performed in the rat pups at P25-P40. The hippocampal protein expression was ascertained by western blot analysis.

Results: Voluntary wheel running reversed maternal restraint stress-induced the expression of β-TrCP in the hippocampus of the pups at P40.

Conclusions: Physical exercise has high potential to ameliorate the adverse effect of maternal restraint-induced prenatal stress at the molecular level. Our results suggested that exercise can ameliorate these adverse effects in the individual who has the vulnerability to prenatal stress.

Keywords: Maternal restraint stress, Voluntary wheel running, β-TrCP, Hippocampus

Introduction

Hippocampus is the brain region that is involved in the modulation of anxiety (1) and essential in the cognitive process (2, 3). Hippocampal neurogenesis is influenced by glutamate, the major excitatory neurotransmitter in the brain. However, neurons overexposed to glutamate have been lead to neurotoxicity (4). Intrauterine milieu plays an important role to regulate fetal brain development. Several lines of evidence have rendered that GCs exposure and prenatal stress concern to impairment of the ability to regulate stress responses and disruption of cognitive processes in the pup rats. The previous study of Son and colleagues induced prenatal stress in the pregnant rats by repeated restraint stress during mid- to late pregnancy. They found that prenatal stress caused the reduction of NMDA receptor subunits and decreased in NMDA receptor-mediated long-term potentiation in the CA1 area of the hippocampus (5). This implies the involvement of prenatal stress in glutamate-induced impairment of learning and memory in the offsprings. Likewise, the repeated corticosterone injection elevated the extracellular glutamate concentration in the hippocampus of the rats (6, 7).

Several lines of evidence revealed that prenatal stress decreased either the number of NMDA receptor subunits or PSD-95 (5, 8, 9). NMDA receptors are anchored onto the surface membrane by interacting with PSD-95 which plays the role of synaptic maturation. SPAR plays an important role to stabilize the surface NMDA receptor by recruitment F-actin to PSD-95 (10-12). The depletion of SPAR is caused by Ubiquitin proteasome system (UPS). β-TrCP is an E3 ubiquitin ligase enzyme which functions to recognize the target proteins. This in turn tagged with ubiquitin and degraded thereafter. Our previous study revealed whether prenatal stress decreased the number of NR2A and NR2B subunits of NMDA receptor, PSD-95 and SPAR but increased in the expression of β-TrCP (8).

Voluntary exercise has benefitted to increase the number of cell proliferation, increased in BDNF mRNA expression in dentate gyrus and improved object recognition memory in rodents (13). Exercise is an interesting intervention to improve prenatal stress-induced impairment of cognitive processes. Postnatal exercise has been reported to alleviate hippocampal neuron apoptosis (14) and improved the impairment of spatial learning ability related to enhancing hippocampal neurogenesis in the prenatal stress in rats (15).

Thus, we hypothesized that postnatally voluntary wheel running exercise may have an efficacy to ameliorate the adverse effects of prenatal stress. In the current study, we aimed to investigate whether exercise influences on the expression of β-TrCP in the hippocampus of the young adult pup rats.

Materials and Methods

Animals

Pregnant Sprague Dawley rats, weighing 270–280 g and their offspring were used in this experiment. Rats were obtained from the National Experimental Animals Center of Mahidol University, Salaya, Thailand and housed in single housing condition in a temperature- and humidity- controlled environment and maintained on a 12-h light/dark cycle with free access to food and water. Each pregnant female was a weight on Gestation day (GD) 7-21 before any other manipulation. On the morning of GD 21, each pregnant female received nesting material, and thereafter, the cage was checked twice daily for the appearance of a litter. The day a litter was discovered will be designated as postnatal

day 0 (P0) and the length of gestation was noted. All experiments conducted according to the Guidelines for Care and Use of the Laboratory Animals and approved by the Experimental Animal Ethics Committee of The Institute of Science and Technology for Research and Development, Mahidol University, Thailand. Every effort was taken to minimize the number of animals used and their suffering.

The rat pups from control and restraint stress rats were grouped into 4 groups as follows; 1) Control group, 2) Control-Exercise group, 3) Stress group and 4) Stress-Exercise group, N=5 for each group.

Maternal restraint stress

For restraint stress, each pregnant rat was put into a small Plexiglas cylindrical cage, in which the diameter and length can be adjusted to accommodate the size of each animal. The restraint stress was performed during GD14-21, four hours daily during the dark phase of the cycle. The control rats were left undisturbed in their home cages. Gestation days 14-21 were selected because this is the most sensitive period to behavioral teratogenic effects of prenatal stress (16).

Voluntary wheel running exercise

The rat pups were trained by housing in the cage with plastic running wheels after weaning on P21-24. The running wheels with 100 cm in diameter were equipped with the recorder for the timing and rotation of the wheel. Wheel running activity was daily recorded at 10 a.m., the pups with less than 100 m running were excluded. The running tests were performed during on P25-P40.

Tissue preparation

The whole hippocampal tissues were collected from rat pups at P40. Brain tissues then were suspended in lysis buffer composed of 50 mM Tris pH 7.4, 150 mM NaCl, 1mM EDTA, 0.5% Na Deoxycholate, 1% SDS, 1 mM PMSF, 1% Triton-X-100 and supplemented with complete protease and phosphatase inhibitor cocktail set (Calbiochem, Germany), then homogenized for 10 sec and centrifuged at 14,000 rpm at 4°C for 15 min. The supernatant was collected for protein determination. The protein concentration of each sample was determined by Bradford protein assay.

Western blot analysis

Protein samples (10 μg) were mixed in a sodium dodecyl sulfate (SDS) sample buffer, and denatured at 100 °C for 5 min. Equal amounts of extracted protein were resolved in a 7–10% SDS–PAGE and underwent electrophoresis at 150 V for 1 h. The protein bands were then transferred to nitrocellulose membrane (Amersham Bioscience, Piscataway, NJ, USA). The transfer efficiency was checked by Ponceau-S red staining. Membranes were washed with Tris-buffered saline (TBS) for 5 min, and then incubated in blocking buffer for 1 h at room temperature. After that, the membranes were incubated overnight at 4 °C with the following primary antibodies: rabbit polyclonal anti-β-TrCP (sc-33213, 1:500) from Santa Cruz Biotechnology, USA, and mouse polyclonal anti-β-actin (AB3563, 1:5000) from Chemicon International, USA. The membranes were then washed 3 times with 0.1% Tween TBS for 5 min each and incubated with the appropriate HRP-conjugated secondary antibodies for 1 h at room temperature, and then washed 3 times with 0.1% Tween TBS for 5 min each. Finally, the signals were visualized using ECL reagent (Amersham Biosciences, Piscataway, NJ, USA) and the immunoreactive bands were developed on X-ray films (Kodak, Rochester, NY, USA). The films were then scanned and digitally processed using Adobe Photoshop software. Sets of

the specific immunoreactive bands on each individual image were quantified by Scion image program (National Institutes of Health, Bethesda, MD, USA). The density of each band was neutralized by the density of β-actin as the internal control.

Data and statistical analysis

Quantitative results were expressed as Mean ± SEM calculated from the duplicate experiments. The statistical significance of difference between the means was evaluated using Student's t-test (unpaired, unless otherwise stated). The probability level of $\mathbf{p} \leq 0.05$ was considered statistically significant difference between the two sets of data. The data were statistically analyzed using GraphPad Prism software.

Results

Repeated maternal restraint stress induced an increase in β-TrCP in the PS group.

First, we examined whether repeated maternal restraint stresses can alter the expression levels of β-TrCP in the hippocampus of the rat pups at P40 (Fig.1). The western blot analysis was performed to compare between stress group and control group. We found that repeated maternal restraint stress induced a significant increase in β-TrCP of stress group when compared to the control group (**p<0.01**).

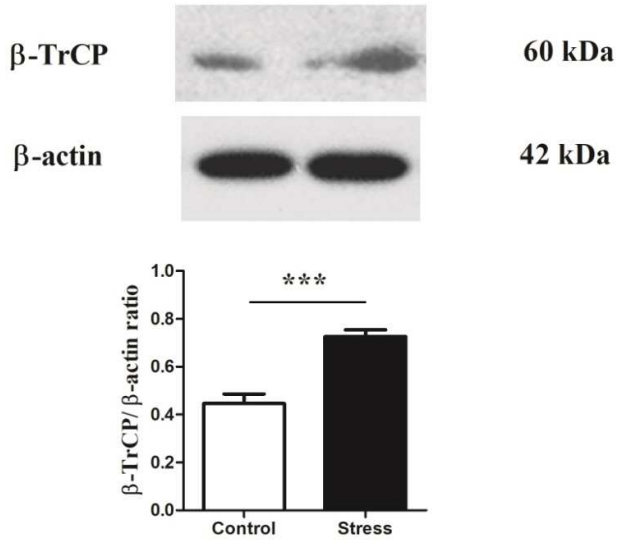

Fig 1. Effects of maternal restraint stress on the levels of β-TrCP in the hippocampus of rat pup at P40. The (Upper) western blot analysis of β-TrCP in the hippocampal tissue compared between stress group and control group at P40. The (Lower) bar graph displays the results from the western blot analysis. The data were expressed as band densities/ β-actin ratio; values represent Mean SEM, N=5. There was a significant difference when compared with the control group at ***p<0.01.

Voluntary wheel running exercise does not attenuate β-TrCP in the hippocampus of pup rats from the non-restraint stress mother rats

Physical exercise enhanced the level of cortisol in plasma. We further determined the effect of voluntary wheel running exercise on the expression of β-TrCP in the pups from the non-restraint stress mother rats (control-exercise group) was ascertained by Western blot analysis and compared with the sedentary control group (Fig.2). The results showed that no significant difference of β-TrCP in the hippocampus of the rat pups at P40 between the control and control-exercise groups. This implied that exercise did not impact the level of hippocampal β-TrCP in the pup rats at P40.

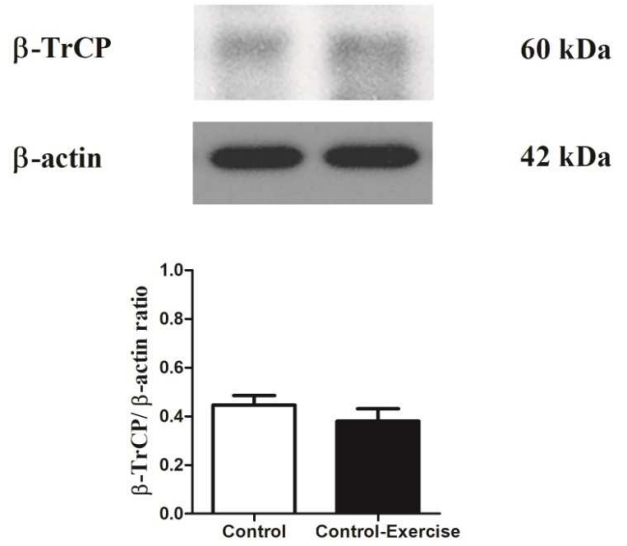

Fig 2. Effects of voluntary wheel running exercise on the levels of β-TrCP in the hippocampus of rat pup at P40. The (Upper) western blot analysis of β-TrCP in the hippocampal tissue compared between control group and control-exercise group at P40. The (Lower) bar graph displays the results from the western blot analysis. The data were expressed as band densities/ β-actin ratio; values represent Mean SEM, N=5.

Voluntary wheel running exercise ameliorated PS-induced an increase in β-TrCP.

To further examine the effect of voluntary wheel running exercise on repeated maternal restraint stress-induced an increase in the level of β-TrCP in the hippocampus of the rat pups at P40 (Fig.3). The results showed that voluntary wheel running exercise significantly decreased β-TrCP in the pup hippocampus of the stress-exercise group as compared to control group (**$p<0.01$**). There's no significant difference in the level of β-TrCP when comparing between the stress-exercise group and control group.

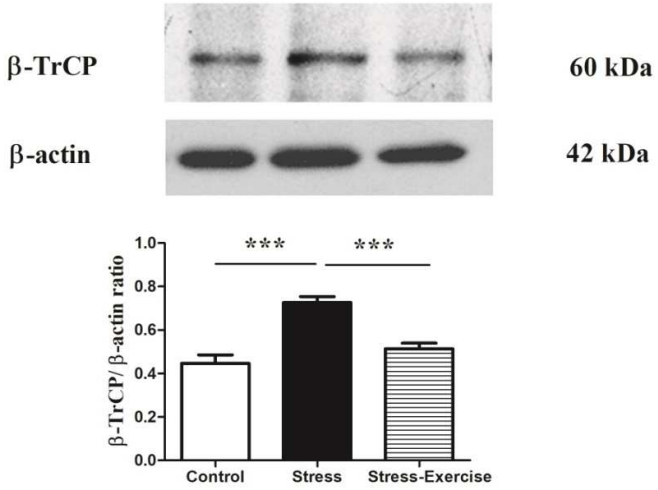

Fig 3. Effects of voluntary wheel running exercise on the levels maternal restraint stress-induced an increase in the level of β-TrCP in the hippocampus of rat pup at P40. The (Upper) western blot analysis of β-TrCP in the hippocampal tissue compared between control group, stress group and stress-exercise group at P40. The (Lower) bar graph displays the results from the western blot analysis. The data were expressed as band densities/ β-actin ratio; values represent Mean SEM, N=5. There was a significant difference when compared with the stress group at ***p<0.01.

Discussion

Repeated maternal restraint stress has a plethora of the adverse effects on the fetal brain development. External environment refined glutamate receptors and their scaffolding proteins, including PSD-95 and SPAR (8, 17). The latter participated in the Snk-SPAR pathway. Serum inducible kinase or Snk plays role to phosphorylate SPAR, which in turn recognized by β-TrCP and degraded by 26s proteasome, there after (4). Our previous study showed that maternal restraint stress decreased the levels of PSD-95 and SPAR in the hippocampus of the rat offspring. Inversely, the increases in the levels of Snk and β-TrCP were shown (8). E3 ubiquitin ligase was responded to several neurodevelopmental disorders, including Autism spectrum disorders (18). Current study, we redered that maternal restraint stress increased the level of β-TrCP. Hence, we aimed to determine the effect of voluntary wheel running exercise on the hippocampal β-TrCP. The increasing evidence rendered that physical exercise positively adjusted either the cellular or the molecular levels in the hippocampus (19). Although, exercise enhanced the level of plasma cortisol, we found that it did not impact to the level of β-TrCP in the offspring from non-stressed mother group. Of interest, our data showed that voluntary wheel running during P25-P40 reversed the effect of maternal restraint stress. The reduction of β-TrCP inversely related to the increases in the levels of PSD-95 and SPAR (the data were not shown.). Recent study postulated that voluntary wheel running ameliorated the symptoms of mental disorders and elevated the expression of BDNF, as well (20).

Conclusions

In conclusion, our data rendered that physical exercise has high potential to ameliorate the adverse effect of maternal restraint-induced prenatal stress at the molecular level. Our results suggested that exercise in the individual who has the vulnerability to prenatal stress, can ameliorate these adverse effects.

Acknowledgement

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