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Effects of treadmill exercise on hypoactivity of the hypothalamo-pituitary-adrenal axis induced by chronic administration of corticosterone in rats

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Abstract

The stress response alters behavior, autonomic function and secretion of multiple hormones, including CRF, ACTH, and glucocorticoid, through the HPA axis. Consecutive stress exposures lead to HPA axis dysregulation such as hyperactivity in Alzheimer's disease and depression, and hypoactivity in post-traumatic stress disorder. In the present study, we established a model of hypoactivated HPA axis in rat through chronic administration of corticosterone (40 mg/kg, s.c.) for 19 consecutive days. In this model, CRF mRNA expression in the hypothalamus and ACTH levels in serum were significantly decreased by chronic administration of corticosterone. In addition, the effect of treadmill exercise was investigated in our hypoactivated HPA axis rat model. Treadmill exercise recovered the dysregulated hypoactivity of the HPA axis induced by corticosterone administration for 19 days. The results of the present study suggest that treadmill exercise may aid recovery of hypoactivated HPA axis dysregulation in psychological diseases such as post-traumatic stress disorder.

Keywords: Treadmill exercise; HPA axis; CRF; ACTH; Corticosterone

Although difficult to fully define, stress is a response to hostile conditions including major life events and the problems of daily life. Initiation of various stress responses improves the ability of an organism to adjust its homeostasis and increases the probability of survival [5]. The stress response activates the hypothalamic–pituitary–adrenal (HPA) axis, which affects behavior and peripheral changes [6]. The stress response in the neuroendocrine system includes release of corticotropin-releasing factor (CRF) from the hypothalamic paraventricular nucleus and the secretion of pituitary adrenocorticotropin hormone (ACTH), leading to secretion of glucocorticoid (GC) hormones by the adrenal cortex, mainly of corticosterone by the HPA axis [3].

Major alterations in neuroendocrine response are manifest after disruption of the HPA axis by chronic stress [16]. Repetition of a stressful experience with a stressor generates habituation or diminution of the HPA axis responses [2,10,13,15,19,24]. The stressor thus dysregulates the HPA axis, such as in post-traumatic stress disorder (PTSD) [26,27] with resultant hypoactivity of the HPA axis or in Alzheimer's disease and major depression with resultant hyperactivity of the HPA axis [1,8].

In the present study, we established a rat model that mimics the suppression of the HPA axis through chronic administration of corticosterone (CTS). This model might be useful in studying the effects of treadmill exercise (TM) on suppressed HPA axis. Thus, we investigated CRF mRNA expression in the hypothalamus and ACTH concentration in serum to assess the status of hypoactivity of the HPA axis induced by chronic administration of CTS. Also, the effect of treadmill exercise upon the hypoactivated HPA axis was investigated.

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Eighteen male Sprague–Dawley rats (5-week-old) were purchased from Orient Bio (Seoul, Korea) and acclimated for 1 week. Rats were housed four per cage and had free access to food and water. Rats were maintained under standard housing conditions: 12-h light:12-h dark cycle, 22 ± 2 °C. All procedures were performed according to the *Guide for the care and use of laboratory animals*, published by the U.S. National Institutes of Health.

Prior to the treadmill exercise experiments, all rats were allowed to run on a motorized treadmill at a low speed (9 m/min) for 10 min for 5 consecutive days to adapt to running on the motorized treadmill. After that, open-field (100 cm W × 100 cm L × 45 cm H) tests were carried out using Any-maze (Stoelting, IL, USA), a behavioral video tracking system, to examine basal locomotor activity. The mean number of line crosses and center line crosses was measured for 5 min. Rats that had equivalent locomotor activity were appropriately assigned to groups following the data analysis of the open-field tests. Finally, 12 rats were assigned to sedentary groups (Control group, $n = 6$; CTS group, $n = 6$) and 6 rats were assigned to the treadmill exercise (TM) group for further experiments.

The six rats in the exercise group were trained for 30 min/day on the motorized treadmill for 19 consecutive days. The training speed ranged from 12 to 15 m/min. The 6 rats in the control group and the 6 rats in the CTS group (collectively the sedentary group) were injected subcutaneously with saline and a dosage of 40 mg/kg of corticosterone (Sigma) daily for 19 days, respectively. The 6 rats (CTS + TM group) in the exercise group were also given CTS treatment.

Rat body weights were recorded daily during the experiment. After the final injection and treadmill exercise, animals were anesthetized with ether, and serum was collected from the heart with a Vacutainer SST gel and Clot Activator tube (BD Bioscience). Brains were also collected via surgery. All samples were immediately frozen in liquid N_2 and then stored at −80 °C.

Total RNA was isolated from rat hypothalami in each group using the easy-BLUE™ Total RNA extraction kit (Intron, Korea) according to the manufacturer's instructions. Total RNA was reverse transcribed by adding 1 μg of total RNA to a mixture containing 1 μL of anchored primer $(dT)_{25}V$, 8 μL of DEPC-treated sterile water, 2 μL of 100 mM DTT, and 8 μL of RT-&GO™ MasterMix (QBIOgene, USA). All samples were incubated at 42 °C for 1 h, and the reverse transcriptase was then inactivated at 70 °C for 15 min in order to prevent inhibition of Taq DNA polymerase by reverse transcriptase. The cDNA was analyzed by real-time PCR with the rat CRF primer set (forward primer sequence: tgatccgcatgggtgaagaatacttcctc, reverse primer sequence: cccgataatctccatcagtttcctgttgctg) and SYBR Green in the ABI 7900 HT (Applied Bioscience, USA). The real-time PCR protocol consisted of 30 s at 95 °C, 30 s at 60 °C, 30 s at 72 °C for 40 cycles.

The ACTH enzyme immunoassay (EIA) kit was purchased from Peninsular Laboratories, Inc. (CA, USA). Prior to use, peptides in rat serum were filtered with 10,000 MWCO centrifugal filter device (Vivascience, Germany). For the competitive enzyme immunoassay, peptide antibody and non-biotinylated peptide including 50 μL of a standard (0, 0.016, 0.08, 0.4, 2.0, and 10 ng/mL) or unknown sample were placed in a well and mixed. 25 μL of primary antisera were incubated for 1 h at room temperature to bind to the specially-treated walls of the well. After preincubation of peptide antibody and non-biotinylated peptide (either standard or unknown), 25 μL of biotinylated peptide solution was added to each well. The biotinylated peptide was then allowed to compete for the antibody binding sites with the standard peptide or the unknown sample peptide for 2 h at room temperature. After incubation, unbound biotinylated and non-biotinylated peptide were removed by washing 5 times with assay buffer. 100 μL of the diluted streptavidin-conjugated horseradish peroxidase (SA-HRP) solution was then added and allowed to bind to the immobilized primary antibody/biotinylated peptide complex for 1 h at room temperature. After 5 washes to remove excess SA-HRP, 100 μL of 3,3′,5,5′-tetramethyl benzidine (TMB) dihydrochloride was reacted with the bound HRP for 10 min at room temperature. The color intensity that developed depended on the quantity of biotinylated peptide bound to the immobilized antibody. 100 μL of 2N HCl was added to terminate the reaction, and the optical density was read at 450 nm using the VERSAmax microplate reader (Molecular Devices, USA).

Data are expressed as the mean and standard deviation. Statistical differences between groups were determined by LSD post hoc tests for body weight and food intake and by one-way analysis of variance (ANOVA) for CRF mRNA expression and ACTH concentration. A p-value less than 0.05 was considered statistically significant.

The rats' body weights were determined daily. Despite the assignment of equivalent body weights to each group at the beginning of the experiments, the CTS and CTS + TM groups tended to lose weight (31.7% in CTS and 30.2% in CTS + TM at day 19) during the CTS treatment (Fig. 1A). Food intake was also decreased in both CTS and CTS + TM groups (Fig. 1B). The maximum differences in food intake were 40.9% in CTS at day 19 and 32% in CTS + TM at day 14 compared with the control group. These results indicate that chronic administration of CTS (40 mg/kg, s.c.) induces loss of body weight that might be influenced by decreased food intake.

To investigate the hypoactivity of the HPA axis by chronic administration of CTS in rats, CRF mRNA in hypothalamus and ACTH levels in serum were measured by qRT-PCR and EIA, respectively. CRF mRNA expression in the CTS group was significantly less (42%) than in the control group (Fig. 2). ACTH concentration in CTS was also less (40.7%) than in control (Fig. 3). In contrast, CRF mRNA (Fig. 2) and ACTH concentration (Fig. 3) were not significantly changed in the CTS + TM group. Both the expression of hypothalamic CRF mRNA and the level of serum ACTH in the CTS + TM group were significantly elevated compared with the CTS group. The present results indicate that chronic administration of CTS (40 mg/kg, s.c.) for 19 consecutive days suppressed the HPA axis in rat brain. Also, treadmill exercise recovered the hypoactivity of the HPA axis induced by CTS administration.

Chronic stress usually reduces food intake in male rats [4]. Decreased body weight gain and food intake are typical phenomena in the dysregulation of HPA axis when glucocorticoid is