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Exercise Training Plus Calorie Restriction Causes Synergistic Protection against Cognitive Decline via Up-regulation of BDNF in Hippocampus of Stroke-prone Hypertensive Rats

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Abstract— **One of the important organ damage of hypertension is cognitive decline. Cognitive function is determined by the function of hippocampus, and previous studies have suggested that the decrease in brain-derived neurotrophic factor (BDNF) in the hippocampus causes cognitive decline. Protection against cognitive decline is reported not only in pharmacological therapy but also in exercise training or calorie restriction. The aim of the present study was to determine whether exercise training plus calorie restriction cause synergistic protection against cognitive decline via BDNF in the hippocampus or not. Exercise training for 28 days improved cognitive decline determined by Morris water maze test via up-regulation of BDNF in the hippocampus of stroke-prone spontaneously hypertensive rats, whereas calorie restriction for 28 days did not. However, exercise training plus calorie restriction causes the protection against cognitive decline to a greater extent than exercise training alone. In conclusion, exercise training plus calorie restriction causes synergistic protection against cognitive decline via up-regulation of BDNF in the hippocampus of stroke-prone hypertensive rats.**

I. Introduction

One of the important organ damages of hypertension and cardiovascular diseases is cognitive decline. Systemic oxidative stress and/or antioxidant deficiency cause cognitive decline [1], and especially, oxidative stress in hippocampus impairs cognitive function [2]. In the brain, brain-derived neurotrophic factor (BDNF) is known to be involved in the protective mechanisms against stress and cell death as an antioxidant [3-5]. In the hippocampus, BDNF protects against ischemic cell damage [6].

Not only the pharmacological therapy but also exercise training [7-9] or calorie restriction [10. 11] has been suggested to cause the protection against cognitive decline. Furthermore, calorie restriction improved cognitive function through the effects on hippocampus. However, in a previous clinical study, calorie restriction and/or exercise training did not protect against cognitive decline [12]. In hypertensive rats, it has not been determined whether calorie restriction protects against cognitive decline or not. The mechanisms in which exercise training and/or calorie restriction cause the protection against cognitive decline should be discussed more.

The aim of the present study was to determine whether exercise training plus calorie restriction causes synergistic protection against cognitive decline via up-regulation of BDNF in the hippocampus of stroke-prone hypertensive rats. To do this aim, we used stroke-prone spontaneously hypertensive rats (SHRSP), as hypertensive and vascular dementia model rats [13]. We divided SHRSP into 4 groups, SHRSP with exercise training (EX), SHRSP with calorie restriction (CR), SHRSP with exercise training plus calorie restriction (E+C), and control SHRSP (Ctl). Exercise training and/or calorie restriction were done for 28 days. Cognitive function was determined by Morris water maze test.

II. Methods

A. Animals

This study was reviewed and approved by the committee on ethics of Animal Experiments, Kyushu University Graduate School of Medical Sciences, and conducted according to the Guidelines for Animal Experiments of Kyushu University. Male SHRSP (12 to 14 week old), weighing 350 to 425 g and fed standard feed were used (SLC Japan, Hamamatsu, Japan). They were housed individually in a temperature-controlled room (22° to 23°C) with a 12-hour/12-hour light-dark cycle (lights on at 7:00 AM). We divided SHRSP into 4 groups, EX, CR, E+C, and Ctl (n=5 for each). Systolic blood pressure was measured dairy using the tail-cuff method (BP-98A; Softron, Tokyo, Japan).

B. Exercise Training

EX and E+C groups were submitted to a maximal exercise test on the treadmill (20 degree angle, 10 m/min for 30 minutes) every day for 28 days, as previously described [14].

C. Calorie Restriction

CR and E+C groups were given 70% of their mean 24-hour food intake. Food was given dairy 2-3 hours before lights off. EX and control groups were free to have food, as previously described [15].

D. Western Blotting Analysis

At the end of the protocol, to obtain the hippocampus tissues, the rats were deeply anesthetized with sodium pentobarbital (100 mg/kg IP) and perfused transcardially with PBS (150 mol/L NaCl, 3 mmol/L KCl, and 5 nmol/L

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phosphate; pH 7.4, 4°C). The brains were removed quickly, and sections 1 mm thick were obtained with a cryostat at –7±1°C. The hippocampus defined according to a rat brain atlas and obtained by a punch-out technique, and the hippocampus tissues were homogenized and then sonicated in a lysing buffer containing 40 mmol/L HEPES, 1% Triton X-100, 10% glycerol, and 1 mmol/L phenylmethanesulfonyl fluoride. The tissue lysate was centrifuged at 6000 rpm for 5 minutes at 4°C with a microcentrifuge. The lysate was collected, and protein concentration was determined with a BCA protein assay kit (Pierce). An aliquot of 20 μg of protein from each sample was separated on 12% SDS-polyacrylamide gel. Proteins were subsequently transferred onto polyvinylidene difluoride membranes (Immobilon-P membrane; Millipore). Membranes were incubated for 2 hours with a rabbit polyclonal antiserum against BDNF (1:1000; Abcam, Cambridge, UK) or α-tubulin (1:1000; Cell Signaling). Membranes were then washed and incubated with a horseradish peroxidase–conjugated horse anti-mouse IgG antibody (1:10000) for 40 minutes. Immunoreactivity was detected by enhanced chemiluminescence autoradiography (plus Western blotting detection kit; Amersham), and was expressed as the ratio to β-tubulin protein.

E. Morris Water Maze Test

Spatial leaning and memory function of the rats were investigated with the Morris water maze test in a circular pool filled with water at a temperature of 25.0±1°C [16]. In the hidden platform test, a transparent platform was submerged 1cm below the water level. Swimming paths were tracked with a camera fixed on the ceiling of the room and stored in a computer. All the procedures of the Morris water maze were performed for 7 days. A pre-training session was carried out at day 0, in which animals were given 60 seconds free swimming without the platform. In the hidden-platform test for 4 days, the rats were given 2 trials (1 session) on day 1 and 4 trials (2 sessions) per day on day 2, 3, and 4. The initial trial interval was about 30 min and the inter-session interval was 2 hours. During each trial, the rats were released from four pseudo-randomly assigned starting points and allowed to swim for 60 seconds. After mounting the platform, the rats were allowed to remain there for 15 seconds, and were then placed in the home cage until the start of the next trial. If a rat was unable to find the platform within 60 seconds, it was guided to the platform and allowed to rest on the platform for 15 seconds. Probe trials were performed at day 5. In the probe trial, the hidden platform was removed and the rats was released from the right quadrant and allowed to swim freely for 60 seconds. The time spent in the target quadrant, where the platform has been located during training, and the time spent in the other quadrants were measured. In the visible-platform test was performed at day 6, the platform was elevated above the water surface and placed in a different position. The rats were given for trials with an inter-trial interval of 10 minutes.

F. Statistical Analysis

All values are expressed as mean ± SEM. Comparisons between any two mean values were performed using Bonferroni's correction for multiple comparisons. ANOVA was used to compare all the parameters in all groups. Differences were considered to be statistically significant at a P value of <0.05.

III. RESULTS

A. Blood Pressure

Systolic blood pressure was reduced to the similar levels in EX and E+C, and was significantly lower in EX and E+C than in Ctl (Fig. 1). Systolic blood pressure was not different between in CR and Ctl (Fig. 1).

B. BDNF in the Hippocampus

The expression of BDNF in the hippocampus was significantly higher in E+C than in Ctl to a greater extent than in EX (Fig. 2). However, the expression of BDNF in the hippocampus was not different between in CR and Ctl (Fig. 2).

C. Morris Water Maze Test

In the hidden platform test, escape latency was significantly lower in E+C than in Ctl to a greater extent than in EX (Fig. 3A). However, escape latency was not different in CR and Ctl (Fig. 3A). In the probe test, E+C resulted in significantly more time in the target quadrant as compared with EX, CR, and Ctl (Fig. 3B). In the visible platform test, there were no significant differences in escape latency among all of the groups.

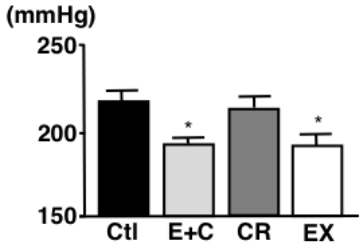

Figure 1. Systolic blood pressure in each groups. *P<0.05 versus Ctl, n=5 for each. Abbreviations; Ctl, control; E+C. exercise training+calorie restriction; CR, calorie restriction; EX, exercise training.

IV. DISCUSSION

In the present study, we demonstrated that exercise training plus calorie restriction improves cognitive performance and increases BDNF in the hippocampus of SHRSP to a greater extent than exercise training alone. However, calorie restriction alone did not have such effects.

These results suggest that exercise training plus calorie restriction might cause synergistic protection against cognitive decline via up-regulation of BDNF in the hippocampus of SHRSP.

It has already demonstrated that exercise training cause the protection against cognitive decline via up-regulation of BDNF in the hippocampus [7, 8]. The results obtained in the present study were compatible to these previous studies. A previous study indicates that superoxide induces down-regulation of BDNF via phosphorylation of cAMP response element binding protein [17]. We have demonstrated that angiotensin II type 1 receptor-induced superoxide is increased in the brain of SHRSP [18], and exercise training reduces superoxide in the brain of SHRSP [14]. Several previous studies have demonstrated that the exercise training inhibits the brain renin-angiotensin system including angiotensin converting enzyme (ACE), ACE2, angiotensin II, angiotensin- (1-7), and their receptors [19-21]. Furthermore, one of the important activating factors of brain renin-angiotensin system is the inflammatory cascade [22], and exercise training is known to lower the inflammatory substances in the brain of rats [23]. We consider that the exercise training-induced anti-inflammation, anti-oxidant, and inhibition of brain renin-angiotensin system cause the up-regulation of BDNF in the hippocampus, which contribute to the protection against cognitive decline.

Interestingly, in the present study, exercise training plus calorie restriction improved the cognitive performance and increases BDNF in the hippocampus to a greater extent than exercise training alone in spite of the similar depressor effects, whereas calorie restriction alone did not cause such effects. We consider that these results involve two findings. First, exercise training-induced protection against cognitive decline is independent of its depressor effect. Second, exercise training plus calorie restriction causes synergistic

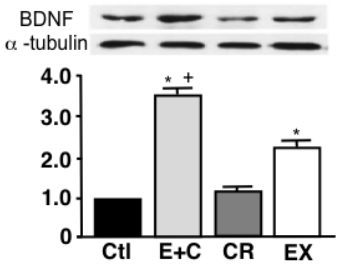

Figure 2. Expression of BDNF in the hippocampus in each group. BDNF / α-tubulin expression was expressed relative to that in Ctl, which was assingned a value of 1. *P<0.05 versus Ctl, +P<0.05 in E+C versus EX, n=5 for each. Abbreviations; Ctl, control; E+C. exercise training+calorie restriction; CR, calorie restriction; EX, exercise training.

protection effect against cognitive decline. Previously, we have demonstrated that exercise training inhibits sympathetic nervous system activation via reduction of oxidative stress in the brain of SHRSP [14]. Furthermore, we also have demonstrated that calorie restriction inhibits sympathetic nervous system activation via reduction of oxidative stress in the brain of dietary-induced obesity rats [15]. These previous results suggest that exercise training or calorie restriction could affect the brain. Although the mechanism in which calorie restriction inhibits oxidative stress in the brain could not been determined in the present study, we hypothesize that calorie restriction may improve adipocytes, inhibit central renin-angiotensin system, directly inhibit oxidative stress in the brain. Circulating angiotensin II acts at circumventricular organs to subsequently activate complex pathways, including those using central angiotensin II as a neurotransmitter, to increases sympathetic outflow [24]. We consider that calorie restriction also reduce oxidative stress in the hippocampus through these mechanisms, and causes the synergistic effect to exercise training. However, it is necessary to do further examination.

To determine the cognitive function, we performed Morris water maze test in the present study, instead of the shuttle avoidance test so that we could focus on hippocampus function. A spatial working memory task, such as Morris water maze test, depends on hippocampus function [25, 26]. Moreover, we used SHRSP as a hypertension and cerebrovascular disease model, and examined the cognitive function by only Morris water maze test. We must do further examination with regard to other cognitive functions in other models, such as Alzheimer, diabetic, and aging.

There are several study limitations in the present study. First, we did not determine the strength and the physiological benefits of the exercise training, such as body weights, lactate level and maximum O_2 consumption. Second, we did not check the calorie restriction-induced changes in metabolism. We did not clarify the cause-and-effect between exercise training/calorie restriction and cognitive function due to these limitations. We have to perform the further studies.

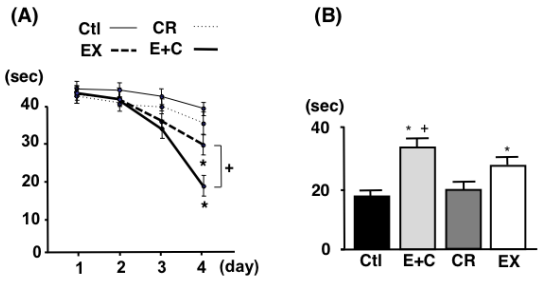

Figure 3. (A) Escape latency time in each group, (B)Time in the target quadrant in each group. *P<0.05 versus Ctl, +P<0.05 in E+C versus EX, n=5 for each. Abbreviations; Ctl, control; E+C. exercise training+calorie restriction; CR, calorie restriction; EX, exercise training.

V. CONCLUSION

Exercise training plus calorie restriction causes synergistic protective effect against cognitive decline via up-regulation of BDNF in the hippocampus of SHRSP. These results indicate that both exercise training and calorie restriction should be done to the patients with hypertension for the protection against cognitive decline in addition to the pharmacological therapy.

APPENDIX

None.

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