Acute bouts of low-intensity prolonged exercise do not change leptin sensitivity in hypothalamus of rats

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Key words : Food intake, Exercise, Leptin sensitivity

Abstract

Leptin, which is secreted from body adiposity, has the potential to decrease the amount of food intake by operating on the hypothalamus. Furthermore, it was reported that vigorous swimming exercise increased sensitivity to the anorectic effects of leptin in hypothalamus of rats. Therefore, it is possible that vigorous exercise could reduce food intake and prevent obesity. Although it is well known that low-intensity exercise, in which the intensity is below lactate threshold (LT) is effective in the prevention of obesity, it is not clear whether low-intensity exercise increases the leptin sensitivity in hypothalamus. Therefore, this study was undertaken to ascertain whether low-intensity exercise (below LT) increases sensitivity to the anorectic effects of centrally administered leptin in rats.

Male rats were exercised using treadmill running at 10m/min for 6 hours. Rats were injected with leptin or saline intracerebroventricularly immediately after the cessation of exercise, and then food intake was measured at 4, 24, 48, 72 and 96 hours after injection. As a result, leptin injection reduced food intake in both control and exercised rats. However, leptin-induced decrease in food intake in exercised rats was not greater than that in control rats. These results suggest that prolonged exercise in which intensity is below LT is not effective for elevating leptin sensitivity in hypothalamus.

Introduction

Leptin is a peptide hormone that is secreted from adipose cells1-2). This hormone increases energy consumption in the body, and has the effect of promoting fat dissolution while it operates on hypothalamus and decreases the amount of food intake. It is known that the amount of leptin secretion will increase following accumulation of body fat. Accordingly, when there is a surplus of body fat (energy), the appetite of an animal is suppressed by the rise of plasma leptin concentration in order to avoid excessive energy intake2-4). On the other hand, appetite is promoted by reduction of plasma leptin concentration. Thus, it can be inferred that leptin is a regulating factor that constantly keeps to body fat.

Previous study has demonstrated that voluntary running exercise prevents hyperphagia and obesity in Otsuka Long-Evans Tokushima fatty (OLETF) rats5). The study demonstrating that voluntary running exercise decreased plasma leptin levels as well as reduced food intake suggests the possibility that the feeding-inhibitory effect of exercise is due to increased sensitivity to leptin’s suppressive effects on food intake in hypothalamus. Furthermore, Flores et al. (2006) reported that 6 hours of forced swimming increased sensitivity to the anorectic effects of centrally administered leptin in rats6). However, the intensity of exercise cannot be defined, when animals exercise on voluntary running wheels or
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When animals are forced to exercise in swimming pools. Since exercise produces multiple physiological effects depending on its intensity and duration, it is important to elucidate the effect of exercise intensity on leptin sensitivity in hypothalamus.

Forced treadmill running is a common method of exercise used by researchers investigating physiological adaptations produced by exercise. One benefit of this specific mode of exercise is that the intensity of the running can be defined. Although the preventive effect of low-intensity exercise (below lactate threshold) against obesity is well known, it is not clear whether low-intensity exercise has the beneficial effect of suppressing food intake. Therefore, we examined whether low-intensity prolonged treadmill running increases sensitivity to the anorectic effects of centrally administered leptin in rats.

Materials and Methods

Animals

In the present experiment, 18 Wistar strain mature male rats (286-342 g body weight) were used. The rats were housed in individual stainless steel cages and were given a standard chow (MF, Oriental Yeast Co., Tokyo, Japan) and water ad libitum under controlled light-dark cycle (light on from 6:00 to 18:00). The room temperature was kept constant at 23°C. The amount of food intake and the body weight of the rats were measured at 18:00 every day during the experiment period.

Surgical procedure

The rats used were anaesthetized with pentobarbital (50 mg/kg body weight, i.p.), and a stainless steel cannula was implanted into the lateral ventricle to a depth of 4.2 mm from the skull surface, 8 mm posterior bregma, and 1.4 mm lateral from the midline, based on the atlas of Paxinos and Watson (2005). After a recovery period of 4 - 8 days, body weight and food intake returned to the level before surgery.

During the recovery period, 40 ng angiotensin II (ANG II, Sigma-ardrich Co., Saint Louis, USA) in 4 μl saline was injected by a 25 μl Hamilton syringe (Hamilton Co., Reno, USA) and a syringe pump (PHD 2000(IW), Haeverd Co., Holliston, USA) into the lateral ventricle in the rats to check whether or not the cannula had been implanted correctly. The injection rate of ANG II was 10 ng/min. Only the rats which drank over 5 ml of water within 30 minutes after the ANG II injection were used for the subsequent experiments.

Experiment procedure

A total number of 18 rats were used for this experiment. These rats were divided into the Non-Exercise (NEx, a total number of 9 rats) group and the Exercise (Ex, a total number of 9 rats) group at random. During the recovery period of 10 - 12 days after ANGII injection, the rats in Ex group were acclimated to treadmill running (15 m/min) for 4 days (15 minutes per day). All rats fasted for 24 hours after the recovery period. At 18 hours after the beginning of fasting, the rats of Ex group (n=6) were forced to run at a speed of 10 m/min for 6 hours using a treadmill (Natsume Seisakusyo Co., Tokyo, Japan). Previous study reported that, when rats run above a speed of 20 m/min on treadmill, the steady state of blood lactate accumulation breaks down. Therefore, 20 m/min of treadmill running corresponds to the lactate threshold (LT; 50 - 70% VO2max) in rats. Thus, 10 m/min of treadmill running which was used in our present study can be considered low-intensity exercise (below LT). Immediately after the exercise, 10 μg of leptin (Sigma-ardrich Co., Saint Louis, USA) dissolved in 2 μl saline were injected into the cerebral ventricle in NEx (n=9) and Ex group rats at a rate of 1 μl/min. The rats were returned to their individual cages just after the injection. The amount of food intake was measured at 4, 24, 48,
72 and 96 hours after leptin injection.

8 - 10 days after leptin injection, NEx (n=8) and Ex (n=8) group rats fasted again for 24 hours. At 18 hours after the beginning of fasting, Ex group was forced to run at a speed of 10 m/min for 6 hours using a treadmill. Immediately after the exercise, 2 μl saline was injected into their cerebral ventricles. The amount of food intake was measured at 4, 24, 48, 72 and 96 hours after saline injection.

Statistical analysis

The amount of food intake and body weight were measured statistically with 2-way analysis of variance (2-way ANOVA). 2-way ANOVA was used to evaluate the main and interaction effect of "Exercise" which was composed of two levels (NEx, Ex), and "Injection" which was composed of two levels (leptin, saline). Post hoc comparisons were performed using Tukey’s test. The significance was assumed to be less than $P < 0.05$.

Results

The food intake at 0 - 4 (Figure 1A), 48 - 72, and 72 - 96 (Figure is not shown) hours after intracerebroventricular (ICV) injection were not apparently different in saline-injected rats with the same exercise conditions. As seen Figure 1B, leptin-injected rats showed a significant reduction of food intake ($P < 0.05$) with the same exercise conditions at 0 - 24 hours after injection. On the other hand, in Figure 1C, leptin-injected rats showed also a significant reduction of food intake ($P < 0.01$) with the same exercise conditions at 24 - 48 hours after injection. However, during all measurement periods after ICV injection, food intakes were not significantly different from the NEx rats with the same injection, respectively (Figure 1).

Discussion

Flores et al. (2006) showed that, after 6 hours of forced swimming, ICV injection of leptin reduced food intake in exercised rats to a greater extent than that observed in control rats$^9$. They also showed that exercise was associated with a markedly increased phosphorylation/activity of several proteins involved in leptin signal transduction in the hypothalamus. This result provides direct evidence that exercise increases leptin sensitivity in hypothalamus. In our present
study, low-intensity prolonged treadmill running did not increase the sensitivity to the anorectic effects of centrally administered leptin in rats. Currently we do not know the reason for this discrepancy, however, it is possible that it could be due to the differences in exercise mode (swimming or treadmill running) and intensity.

Previous studies showed that prolonged vigorous exercise of moderate (50 - 70% VO2max) to high (above 80% VO2max) intensity induces a reduction in food intake\(^9,10\). Although the intensity of swimming exercise that was adopted by Flores et al. (2006)'s study is not clear\(^6\), it is possible that exercise intensity of forced swimming is higher than that of our low-intensity prolonged running. Increased circulating levels of interleukin (IL)-6 have been reported in response to exercise\(^11\). Furthermore, it has been demonstrated that IL-6 is produced in and released from working muscles during exercise\(^12,13\). Flores et al. (2006) also reported that swimming induced increase in hypothalamic leptin sensitivity was blocked by pretreatment with anti-IL-6 antibody\(^6\). Therefore, it is suggested that action of IL-6 that is derived from working muscle enhances the leptin induced anorexic effect in hypothalamus of exercised rats. It was reported that the magnitude of the increase in circulating levels of IL-6 depends on the intensity of exercise\(^13\). If exercise intensity is higher during swimming which was adopted by Flores et al. (2006)'s study\(^6\) than during treadmill running in our present study, blood IL-6 level would be higher during swimming than during treadmill running. This can explain the discrepancy in exercise effect on leptin sensitivity between studies. We are planning to measure blood IL-6 concentration in rats which are exercised in our treadmill protocol in our future study.

In conclusion, when rats were exercised by treadmill running at 10 m/min for 6 hours, intracerebroventricular leptin injection did not reduce food intake in exercised rats to a greater extent than that observed in control rats. Therefore, it is suggested that low-intensity prolonged treadmill running in which intensity is below LT does not increase the sensitivity to the anorectic effects of centrally administered leptin in rats.

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**References**


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