ORIGINAL

Intracerebroventricular injection of adiponectin regulates locomotor activity in rats

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Abstract : Enhancing exercise motivation is the best way to prevent obesity and diabetes. In this study, we examined whether adiponectin affects locomotion activity in Wister and Spontaneously-Running Tokushima-Shikoku (SPORTS) rats using two types of behavioral assays : home cage and wheel running activity. SPORTS rats were established from an original line from Wister strain that had shown high level of wheel running activity in our laboratory. Injection of adiponectin into the lateral ventricle of Wister rats and SPORTS rats decreased home cage activity, but no change was observed in the food intake and oxygen consumption. This result indicates the possibility that adiponectin can reduce non-exercise activity thermogenesis (NEAT) and physical activity via the central nervous system. In contrast, injection of adiponectin did not change wheel running activity in SPORTS rats. We produced hypothalamus-destructed model rat using monosodium glutamate (MSG) to elucidate the regulation site of adiponectin. Injection of adiponectin into MSG-treated SPORTS rats did not change amount of home cage activity and food intake, suggesting that adiponectin action on home cage activity was in the hypothalamic area. These results suggest that adiponectin regulates locomotion activity through mediobasal hypothalamus.
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INTRODUCTION

Obesity and lifestyle-related diseases are major problems faced by people in developed nations. Although exercise training prevents the progression of diabetes and obesity (1), motivation for exercise is generally low in obese animals and humans. Increasing motivation for exercise is the best treatment for obesity because there is a report that education on the importance of exercise training improves motivation level for exercise (2). Increasing non-exercise activity thermogenesis (NEAT), a mechanism by which small amounts of energy is spent has been suggested to prevent weight gain. NEAT is the predominant component of thermogenesis activity, and involves the energy expenditure associated with all the activities except for sports-like exercise, sleeping, and food intake (3). Some neuronal peptides and hormones are also involved in the regulation of locomotion activity as well as food intake and energy metabolism. Orexin/hypocretin, a neuropeptide locally produced in lateral hypothalamic area, elevates locomotion activity and oxygen consumption ; thereby, increasing NEAT, and as a consequence resisting weight gain (4-7). On the other hand, adipocytokines, secreted from white adipose tissue (WAT), play an important role in energy homeostasis. Of these molecules, adiponectin, an adipocytokine secreted hormone, has been known to exhibit anti-diabetic and anti-atherogenic effects (8, 9). Adiponectin, is secreted exclusively by the WAT and has been shown to increase the sensitivity of peripheral tissues to insulin (10). It has been suggested that the decreased plasma adiponectin levels in obesity and type 2 diabetes may relate to insulin resistance (11, 12). Thus, adiponectin and its receptors have been considered as therapeutic targets of new drug development for metabolic syndrome. However, there has been no evidence yet that central adiponectin is involved in locomotion activity and the regulation of NEAT despite adiponectin receptors being expressed in the hypothalamus.

In this study, we examined whether adiponectin had a role on home cage activity and/or wheel running activity either in Wister rats or Spontaneously-Running Tokushima-Shikoku (SPORTS) rats that we established from an original line of the Wister strain that had shown high levels of spontaneous wheel running activity in our laboratory (13). This knowledge may contribute to the establishment of a new strategy for the prevention of obesity and lifestyle-related diseases.

MATERIALS AND METHOD

Animals

We used male Wister rats (SLC, Inc, Shizuoka Japan) and SPORTS rats. At 12 weeks of age, the SPORTS rats have shown almost six times higher running activity than the control Wister rats, and have therefore been used in this study (13). All the rats were housed singly at a constant room temperature of 23±1°C with a 12-h light-dark cycle (lights on at 8 AM), and were fed a standard nonpurified diet (Oriental Yeast, Tokyo, Japan) with food and water available *ad libitum.* This study conformed to the guidelines for the care and use of Laboratory animals of the University of Tokushima Graduate School, Institute of Health Bioscience. All efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments. In addition, we produced destruction model of the mediobasal hypothalamus.

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SPORTS rats received intraperitoneal injection of monosodium glutaminate (MSG) (4 mg/weight (g)) until 3-5 days postnatal. Dose of MSG was selected as previously described (14).

Intracerebroventricular cannulation and injection

The rats were anesthetized by intraperitoneal injection of sodium pentobarbital (100 μl/kg body weight), and a stainless-steel guide cannula was inserted into the lateral ventricle (AP=-1.0 mm, L=+1.5 mm, H=+3.6 mm taken from bregma). The guide cannula was fixed in the skull by adhesive and dental acrylic cement. A stainless-steel dummy cannula was inserted into the guide and kept there until the start of the experiment. After surgery, the rats were allowed to recover for at least one week. The SPORTS rats received the intracerebroventricular injection of vehicle (0.9% saline), 5 μg adiponectin (1 μg/μl) (Enzo Biochem Inc., New York, America) at 10 PM.

Measurement of locomotion activity and Oxygen consumption

We measured two types of locomotion activity : home cage and wheel running activity. Home cage activity was measured by animal movement analysis system (ACTIMO System ; Shintechno, Fukuoka, Japan). Wheel running activity was measured by Wheel running cage (Shinano Ltd, Tokyo, Japan). The data was acquired at 2 h intervals for 48 h. We used Oxymax (Columbus Instruments, USA) to measure oxygen consumption (VO_2). The data was acquired at 12-h intervals for 24 h.

Statistical analysis

Results were expressed as means ± SEM. All data were analyzed by student t-tests and paired t-tests. $P < 0.05$ was assumed to indicate statistical significance.

RESULTS

Adiponectin regulates home cage activity via the central nerve system

Adiponectin provides health benefit by improving insulin resistance by regulating food intake and metabolism (10, 15, 16). In this study, we hypothesized that adiponectin contributes to systemic metabolism by regulating exercise. We found that the injection of adiponectin into the lateral ventricle of the Wister rats decreased home cage activity (Fig. 1A) while there was no significant difference in food intake and oxygen consumption (Fig. 1B, C). These data suggested that adiponectin affects home cage activity without changing food intake and oxygen consumption.

Adiponectin regulates spontaneous activity.

As expected, SPORTS rats showed high level of wheel running activity compared with the Wister rats (13). We examined whether the locomotion activity had any suppressive effect by adiponectin in the wheel running activity of the SPORTS rats. Injection of adiponectin into lateral ventricle of SPORTS rats decreased home cage activity during the dark phase compared with that of saline injection, but wheel running activity did not change compared with that of saline injection (Fig. 2A, B). Food intake and oxygen consumption did not change after injection of adiponectin compared with saline (Fig. 2C, D). From these results, we demonstrated that adiponectin action in the central nervous system suppressed home cage activity, but did not affect wheel running activity.

MSG treatment canceled exercise-suppressive effect of adiponectin.

We examined the exercise regulation site of adiponectin. In keeping with a previous report that injection of monosodium glutamate (MSG) during the neonatal period destroyed mediobasal

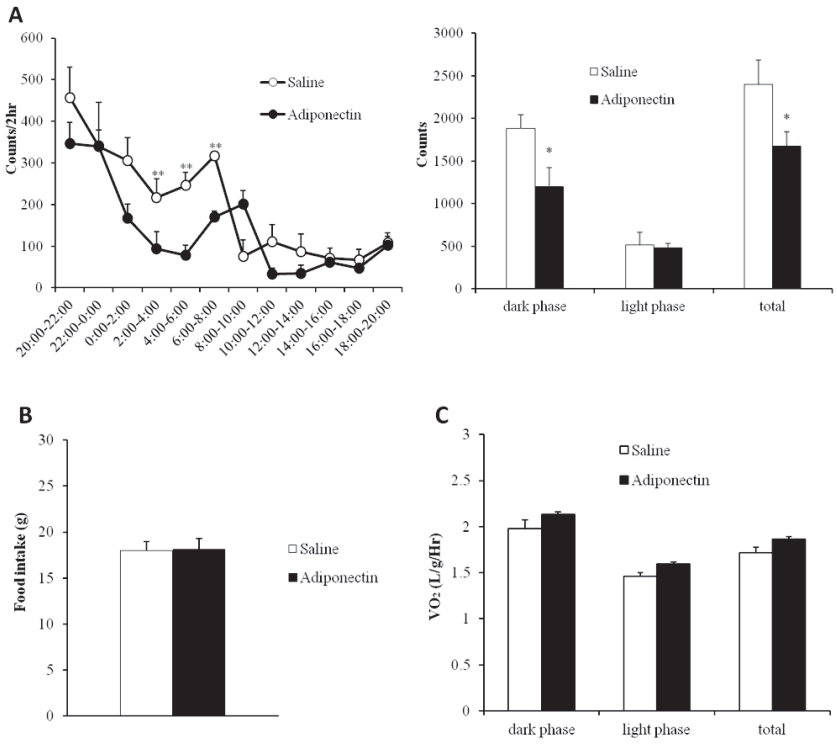

Figure1. Effect of intracerebroventricular injection of adiponectin in a Wister rat
(A) Injection of adiponectin showed suppressive effect of home cage activity in Wister rats (n=4). Values are mean ± SE * $p < 0.05$, * *$p < 0.01$
(B) Injection of adiponectin showed no significant difference of food intake in Wister rats (n=4). Values are mean ± SE (C) Injection of adiponectin showed no significant difference of oxygen consumption in Wister rats (n=4). Values are mean ± SE

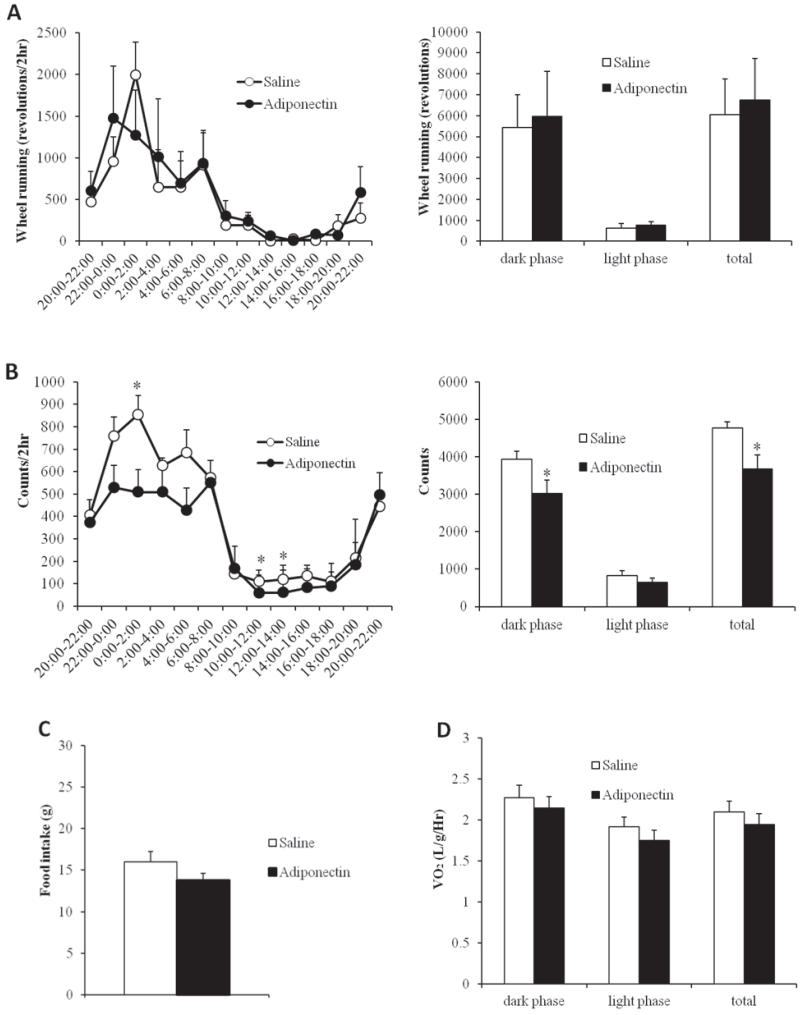

Figure2. Effect of intracerebroventricular injection of adiponectin in a SPORTS rat
(A) Injection of adiponectin showed no significant difference of wheel running activity (n=4). Values are mean ± SE (B) Injection of adiponectin showed suppressive effect of home cage activity (n=4). Values are mean ± SE * $p < 0.05$ (C) Injection of adiponectin showed no significant difference of food intake (n=4). Values are mean ± SE (D) Injection of adiponectin showed no significant difference of oxygen consumption (n=4). Values are mean ± SE

hypothalamus causing increased food intake (17), we also used MSG in our study to destroy the hypothalamus. MSG-treated SPORTS rats showed a high amount of food intake compared with MSG non-treated SPORTS rats (MSG treatment 23.85± 1.36 g, MSG non-treatment 16.01± 1.25 g). Exercise suppressive effect of adiponectin was canceled in MSG-treated SPORTS rats (Fig. 3A). Food intake didn't change even in MSG non-treatment group (Fig. 3B). These results suggested that the regulation of locomotion activity in home cage is through the mediobasal hypothalamus.

DISCUSSION

Adiponectin has been shown to increase the insulin sensitivity in peripheral tissues, and it has been suggested that the decreased plasma adiponectin level in obesity and type 2 diabetes contributed to insulin resistance. Therefore, adiponectin has been known as an anti-diabetic adipocytokine (8). In this study, however, we observed that intracerebroventricular injection of adiponectin in rats decreased locomotion activity in home cage especially in the active phase without significant change in food intake and oxygen consumption. This result indicates a possibility that adiponectin has a function to reduce physical activity and possibly even NEAT via the central nervous system.

We further determined whether adiponectin suppressed the motivation of voluntary wheel running exercise in SPORTS rats, which showed almost two times amount of the home cage activity and six times amount of the wheel running activity compared with control Wister rats (13). We observed that injection of adiponectin into the lateral ventricle in SPORTS rats did not decrease wheel running activity. However, central adiponectin suppressed home cage activity even in SPORTS rats. These results suggest that adiponectin may regulate home cage activity independent to the pathway of voluntary exercise although the critical region in the brain for the motivation of locomotion activity is not clear.

The medial hypothalamus was thought to be the one of the subthalamic locomotor regions in animals (18), while Narita *et al.* reported that chemical stimulation of ventromedial hypothalamus (VMH) in rats enhanced running activity (19, 20). Therefore, we produced a hypothalamus-destruction model rat using MSG to examine whether adiponectin acts on VMH to enhance locomotion activity in home cage. Subcutaneous injection of MSG during the

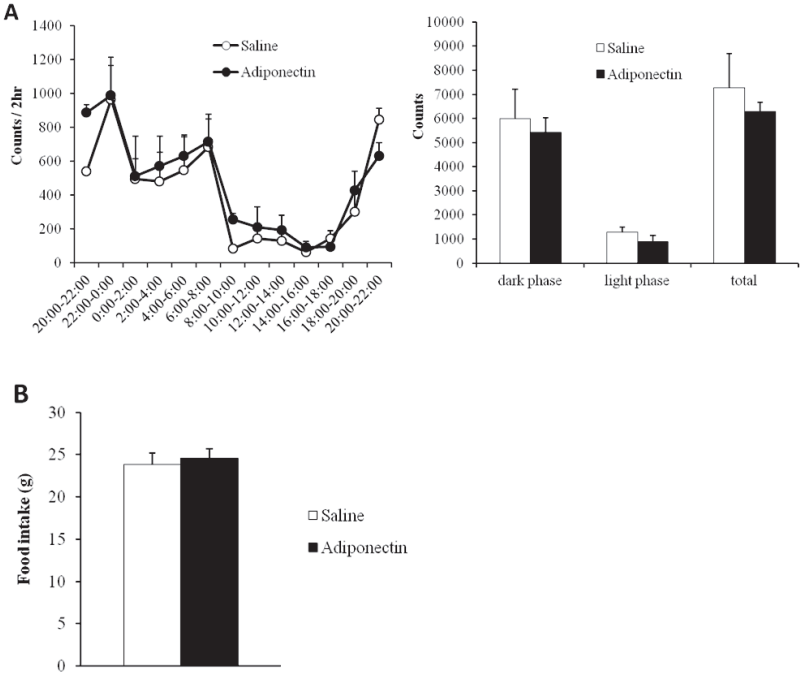

Figure3. Intracerebroventricular injection effect of adiponectin in MSG-treated SPORTS rats
(A) Suppressive effect of home cage by adiponectin was observed and disappeared by intraperitoneal injection of MSG (n=4). Values are means ± SE (B) Injection of adiponectin showed no significant difference of food intake (n=4). Values are means ± SE

neonatal period destroys the mediobasal hypothalamus including VMH (17). The present study showed that suppressive effect of adiponectin on home cage was canceled by the injection of adiponectin into the lateral ventricle in MSG-treated SPORTS rats. However, adiponectin receptors are reported to express in the arcuate nucleus, paraventricular nucleus, and the lateral hypothalamic area in hypothalamus of rodents, while there is no visible evidence that adiponectin receptors are expressed in VMH. Taken together, these findings suggest that adiponectin may regulate home cage indirectly through the mediobasal hypothalamus.

Orexin and hypocretin are synonymous terms, and a typical neuropeptide for the induction of locomotion activity concomitant with food seeking behavior. There are some controversial reports concerning adiponectin-induced feeding behavior (16, 21, 22). Our experiment showed central adiponectin did not affect food intake, neither in the Wister nor in the SPORTS rats. Although this discrepancy may be involved in the injected adiponectin concentration, timing, species, and strains, this consequence indicates that adiponectin action in brain reduces locomotion activity independent of feeding behavior.

Spontaneous activity is regulated by several hormones or peptides (23, 24). A previous study indicated that intracerebroventricular injection of leptin increases wheel running activity (25). We also confirmed that central leptin increased locomotion activity in rats (data not shown). Intracerebroventricular injection of adiponectin showed the opposite effect of leptin with respect to exercise. Intriguingly, leptin also exhibited an opposite action on locomotion activity depending on food availability (26). Further work is needed to precisely define the differential role of central adiponectin on locomotion activity depending on the timing of action of energy events in the whole body.

In conclusion, we showed a novel function of adiponectin on locomotion-suppressive activity via the mediobasal hypothalamus. This effect may work independent of other physical activities such as feeding behavior and voluntary aerobic exercise.

DISCLOSURES

The authors have no conflicts of interest, financial or otherwise, in the publication of this study.

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