Neuroscience Letters 645 (2017) 25–32

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

The effects of intracerebroventricular infusion of irisin on feeding behaviour in rats

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HIGHLIGHTS

- Irisin infusion increased food intake in rats.
- After the irisin infusion, the body weight of rats did not change.
- Irisin infusion decreased the serum leptin level, but the ghrelin level increased.
- Irisin could affects the nutrition behavior and energy metabolism.

ARTICLE INFO

Article history:
Received 7 November 2016
Received in revised form 10 February 2017
Accepted 24 February 2017
Available online 24 February 2017

Keywords:
Body weight
Food intake
Irisin
NPY
POMC
UCP2

ABSTRACT

Irisin, a novel exercise-induced myokine, has attracted attention with its effects on energy metabolism. This study was conducted to determine the possible effects of irisin on nutritional behaviour.

In this study, 40 male Wistar Albino rats were separated into 4 groups (n = 10 for each group). Osmotic mini-pumps were connected to metal cannulas implanted to lateral ventricle; and artificial cerebrospinal fluid (vehicle), and 10 and 100 nM of irisin was infused for 7 days. The daily food and water consumptions and body weights of rats were followed up. After the infusion, the animals were killed, and the hypothalamus and blood samples were collected. NPY, POMC, and UCP2 mRNA levels in the hypothalamus were examined by RT-PCR. In serum, leptin and ghrelin levels as well as the levels of metabolic parameters were measured by using ELISA.

It was determined that irisin administration increased the daily food consumption ($p < 0.05$), without causing significant changes in water consumption and body weight. Irisin also caused increases in ghrelin level in circulation and NPY and UCP2 mRNA levels in the hypothalamus, whereas it decreased the leptin level in circulation and POMC mRNA levels in the hypothalamus ($p < 0.05$). Otherwise, irisin caused decrease in LDL, triglycerides and cholesterol levels, while increasing HDL and glucose levels ($p < 0.05$).

Results indicates that long-term irisin treatment increases food intake without increasing body weight associated with increased ghrelin, NPY and UCP2 mRNAs, and decreased leptin and POMC mRNA in the hypothalamus.

1. Introduction

Food intake is controlled by hypothalamus mainly as a feeding center and is regulated by endocrine and neural signals from the periphery [24]. Energy balance is controlled by two types of neurons in the arcuate nuclei of the hypothalamus: (I) The neurons secreting pro-opiomelanocortin (POMC) and cocaine-and amphetamine regulated-transcript (CART) decrease food intake;

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http://dx.doi.org/10.1016/j.neulet.2017.02.066
0304-3940/

however increase energy expenditure; (II) The neurons producing agouti-related peptide (AgRP) and neuropeptide Y (NPY) increase food intake; however decrease energy expenditure. Therefore, energy expenditure, the regulation of food intake and the amount of energy stored in the body are determined by the susceptibility of the hypothalamus [31]. Uncoupling proteins (UCPs) are expressed within the mitochondrial inner membrane [17]. They play a role in the mitochondrial membrane function and in the cellular energy regulation in response to the glucose [9]. UCP2, a member of the uncoupling protein family, is particularly associated with the hypothalamus. UCP2 is reported to exist densely in NPY and AGRP neurons [4]. Andrews et al. [1] showed that ghrelin had increased the number of UCP2-mediated mitochondria in the hypothalamus.

Irisin, which is idendefined by Bostrom et al. [2] as a peptide myokine, is formed when fibronectin type III domain containing 5 (FNDC5), which has 206 aminoacids released from the muscle tissue during exercises, loses its 94 aminoacids. Some studies suggest that it is produced by cleavage of the FNDC5 receptor; irisin corresponds to the extracellular receptor ectodomain [28]. Moreover, FNDC5-like receptors are highly conserved and have been shown to be critical for neuronal development [13,15,28,33]. But it is currently unknown whether irisin can cross the blood–brain barrier. The FNDC5 gene expression in human tissues shows quite a broad distribution. Along with the study conducted by Huh et al. [16], FNDC5 gene expression was detected in 47 different human tissues, and this protein was determined to exist at quite high levels in the tissues, such as muscles, brain, rectum, intracranial artery and pericardium. It was reported that irisin increased the number of mitochondria in white adipose tissue cells as well as the UCP1 level, maintaining the browning process [2].

In the same study, adenoviral particles that secrete FNDC5 were injected into these rats, and it was demonstrated that there were decreases in the white adipose tissue while the irisin level increased 3–4 times as much, which was accompanied by the development of brown fat cells [2]. It is thought that irisin increases the energy use over UCP1 by affecting the brown fat/adipose tissue and functions as a buffer against obesity. However, the possible relationship between irisin and UCP2 is not fully known. The presence of irisin, which is claimed to increase energy expenditure, within the cerebrospinal fluid (CSF) has also been shown along with a study conducted recently [26]. All these studies suggest that peptide could be effective in the central region and may play major roles in controlling nutritional behaviours. This study was planned for the purpose of investigating the effects of irisin on nutritional behaviours and body weight and also for examining its effects on NPY, POMC, and UCP2 that function in the hypothalamic control of nutrition and on leptin and ghrelin levels that inhibit/activate these neurons in the hypothalamus by taking their origin from the peripheral tissues.

2. Material and method

2.1. Determining the number of rats and assigning them to groups

This study was conducted in the Research and Production Center for Experimental Animals in Inonu University, Faculty of Medicine along with the approval (Dated 02.26.2014; Protocol no: 2014/A-17) received from Animal Experimentation Ethics Committee of Inonu University, Faculty of Medicine. Prior to the commencement of the experiment, the animals were weighed, and their body weights were recorded. In the study, a total of 40 male Wistar Albino rats, the mean weights of which were 240–325 g, were used. The number of animals to be used during the experiments, and the fact that there had to be at least 10 animals in each group in the event that the animals were separated into 4 groups, and also the mean weight of 288 g in initiating the animal experiment, with 22 g standard deviation, 4% deviation, type 1 error (α) 0.05 and type 2 error (β) (Power = 0.80) were all determined through the power analysis. The assignment of animals in groups in accordance with their determined body weights was performed through the simple random assignment method based on computer algorithm (MedCalc 12.7.0 for Windows), and according to the findings of One-Way ANOVA analysis, it was determined that there was no difference among the groups in terms of animal weights before starting the experiment.

The rats were separated into 4 groups as control group, vehicle group, the group to which 10 nM irisin (Catalog#067-16, Phoenix Pharmaceuticals Inc., CA, USA) was administered, and the group to which 100 nM irisin was administered (for each group; n = 10). The rats were individually reserved in single metabolic cages (Tecniplast Metabolic Cage 3700 M, Italy), and the environment where the cages were kept throughout the experimental period was set in the way that it would be at $22 \pm 1\,^{\circ}C$ temperature range and for 12:12 h light-dark period. The rats fed on standard rat feed (Korkutelim Feed and Food Inc., Turkey) as ad libitum and drank normal tap water.

2.2. Procedures performed on the experimental groups and followed-up parameters

The rats in the vehicle group and those in 10 nM and 100 nM irisin administered groups were habituated to the single sheltering environment, and their daily feed and water consumptions as well as their body weights were measured for 7 days, without performing any other process. On the 8th day, Alzet brain infusion kit (DURECT Corporation, ALZET Osmotic Pumps, CA, USA) was implanted in the right lateral ventricles of the rats in these three groups. After the surgical procedure, a 7 days waiting period was given for the recovery of the rats (8th −14th days of the experiment), and during this period, the measurements of their feed and water consumptions as well as their body weights were continued. On the 15th day, osmotic mini pumps (DURECT Corporation, ALZET Osmotic Pumps, CA, USA) were connected to the cannula fixed on the brain infusion kits of the rats, and the vehicle group was administered with intracerebroventricular (icv) artificial CSF (solvent, aCFS; 124 mM NaCl, 5.0 mM KCl, 1.2 mM KH_2PO_4, 2.4 mM $CaCl_2$, 1.3 mM $MgSO_4$, 26 mM $NaHCO_3$, and 30 mM glucose, pH:7.2), whereas the practice groups underwent an infusion of physiological (10 nM/240 μl/day) and pharmacological (100 nM/240 μl/day) concentrations [20] of irisin for 7 days. In order to determine the effect of irisin on nutritional behaviour, the feed and water consumptions of rats as well as their body weights were continued to be followed up. On the other hand, no procedure was performed on the animals in the control group; and throughout 21 days, only their daily feed and water consumptions and body weights were measured.

2.3. Implantation of brain infusion kits

The rats were anaesthetized with the combination of 70 mg/kg ketamine (Richter Pharma AG, Australia) and 8 mg/kg xylazine (Alfasan International B.V., Holland). After the scalps of the rats had been shaved, they were placed in a stereotaxic frame, and the bregma was reached by cutting the scalps. According to Paxinos & Watson's rat brain atlas [25], the bregma was taken as the reference, and the lateral ventricle coordinates (1.40 mm lateral, 0.8 mm posterior, and 4.8 mm vertical) were specified [12,25,27].

This region was drilled with a drilling machine. The cannula of brain infusion kits was filled with aCSF for the vehicle group and with 10 and 100 nM irisin for the irisin groups in the way that there