Irisin injection into the hippocampus suppresses acute stress-induced memory impairment and anxiety-like behavior in a sex dependent manner

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ABSTRACT

Acute stress can change a variety of neural processes, including reducing levels of brain-derived neurotrophic factor (BDNF) in the hippocampus. Exercise, on the other hand, can increase BDNF levels and has overall beneficial effects for health and brain function. Irisin is a myokine that is released into the peripheral blood during aerobic exercise. Although the main known functions of Irisin, both in human and rodents, are forming while adipose tissue and improving glucose homeostasis, recent findings have shown that Irisin mediates the activation of an exercise-induced BDNF-mediated neuroprotective pathway in the hippocampus. Therefore, in this study we revisited the hypothesis that Irisin can counteract the deleterious effects of acute stress when directly injected into the hippocampus. To test our hypothesis, we first established a 5th physical restraint stress in adult mice that resulted in sex-dependent increased anxiety-like behaviors and memory impairment in a combined open field/Novel object recognition (OFNOR) test. Moreover, acute stress also induced skin temperature and body weight in both female and male mice. We then injected Irisin via bilateral stereotactic injection and repeated the acute stress paradigm and combined OFNOR test. We noted that Irisin partially blocked stress-induced anxiety-like behavior and memory impairment in male mice, while also preventing the reduction in skin temperature and body weight. Interestingly, a female mice strain Irisin only prevented the skin temperature and body weight reduction but showed no beneficial effects on neurobehaviors. Taken together, our results support a novel role for Irisin in counteracting acute stress-induced neurobehaviors and physiological abnormalities. Also, our results support the idea that exercise can be a potentially effective tool to promote the maintenance of healthy neural function.

METHODS

EXPERIMENTAL MICE: Adult 8-10 weeks old C57BL/6J mice (Charles River, Wilmington, MA) were group housed (maximum 5 mice per cage) in ventilated cages inside an air-conditioned room. The room was kept at 21-23°C and 40-60% humidity on a 12:12 h light/dark cycle. Mice had ad libitum access to standard laboratory food and water.

ACUTE RESTRAINT STRESS PROTOCOL: For acute restraint stress mice were placed in 20 ml, conical plastic tubes for 10 min. Each tube was partitioned with multiple holes for ventilation and was placed over a water bath inside the experiment room. Control mice were kept in their home cages for the same amount of time.

STEREOTACTIC SURGERY: Human recombinant myokine (Irisin) or vehicle (rat PBS) was injected into the hippocampus by bilateral stereotactic surgery. All injections were performed using the following coordinates: unanesthetized (AP) -2.8 mm, mediolateral (ML) -2.9 mm and dorsoventral (DV) -4 mm coordinates.

BEHAVIORAL TESTS:

Open Field Test (OFT): This test was used to assess locomotion activity and anxiety-like behaviors. This test was performed concurrent with the acquisition phase of the NOIR test. The mice were individually placed in the center area of the arena and allowed to explore for 10 min. The arena was divided in equally sized areas (i.e., center and periphery) and the following parameters were quantified using EthoVision XT 11.1 (Noldus Information Technologies, Inc.): time spent in the center and periphery (both supported and unsupported).

Novel Object Recognition (NOR): We performed NOR test in two phases: acquisition and recognition test, separated by a 24-h rest period. The acquisition phase mice explored two identical objects (“A”) placed in an open-field arena (45×45×30 cm) with the 12 cm diagonal) for 10 min. After 72 h one object was replaced with a novel object (“B”) and mice were placed back and allowed to explore for 10 min. Short-term memory function was measured by quantifying exploratory behavior during the recognition test. Exploration was defined as time in which the mouse was directly interacting with either object (i.e., sniffing, running on or, or directing the nose towards the object). We then calculated a preference index (PI) using the formula: PI = |Time on B / (Time on B + Time on A)|×100.

NON-CONTACT INFRARED TEMPERATURE MEASUREMENT AND BODY WEIGHT MONITORING: A non-contact infrared (IR) thermometer was used to measure surface temperature from the anterior, posterior, and bilateral adipose tissues (BAT). During the “stress” period, thermal images were measured starting at the beginning (T0) and then every 10 min until the end. Change in surface temperature (ΔT) was calculated using formula: ΔT(t)-ΔT(0) at each time point. We monitored mice body weight before acute restraint stress and after by weighing mice using a bench top scale. Then we calculated weight loss percentage by using the formula: Weight loss% = (Weightbaseline - Weighttest) / Weightbaseline × 100.

STATISTICAL ANALYSIS: All statistical analyses were performed using Origin software (OriginLab Corporation). We used Student’s t-test to determine significant differences between two groups. One-way ANOVA, followed by Tukey’s post-hoc test was used for statistical significance between more than two groups. P<0.05 was considered statistically significant.

RESULTS

Acute stress induces anxiety-like behavior in both male and female adult mice

Acute stress induces short-term memory impairment in male mice

Acute stress impacts on exploratory behaviors in both male and female mice

Irisin administration into the hippocampus has no effects on acute stress-induced exploratory behavior impairments

CONCLUSION

Acute stress induces neurobehavioral and physiological impairments differentially in male and female mice. Irisin administration into the hippocampus suppresses acute stress-induced neurobehavioral and physiological impairments in sex dependent manner. Irisin administration into the hippocampus with no exposure into the acute stress has no effects on neurobehaviors and physiological parameters. Irisin could be a potential therapeutic agent for suppressing acute stress-induced impairments.

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