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Short communication

Reproductive effects of irisin: Initial *in vitro* studies

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ABSTRACT

The recently discovered myo- and adipokine irisin affects insulin sensitivity in classical insulin target tissues (adipose tissue, skeletal muscle and liver), but the reproductive effects of this hormone, if any, remain largely unexplored. We hypothesized that irisin may have effects on the hypothalamic-pituitary-gonadal axis. To test this hypothesis, we used murine pituitary mPit12 and human ovarian granulosa cells. GnRH treatment resulted in significant (up to 2.5-fold, $p < 0.0005$) and dose-dependent stimulation of LH production by the mPit12 cells. Treating these cells with irisin alone showed a significant stimulatory effect on LH synthesis only at irisin concentration of 100 ng/ml. When used together with GnRH, irisin abolished the stimulatory effect of GnRH on LH production. Human ovarian granulosa cells were treated with insulin, irisin or a combination of both and the estradiol (E2) production was measured. Both insulin or irisin stimulated granulosa cell E2 production (1.4-fold, $p < 0.05$ and 2.5-fold, $p = 0.0002$, respectively), but when insulin and irisin were used in combination, this stimulatory effect on E2 production was abolished. We conclude that irisin may have reproductive axis effects in the pituitary and in the ovary. Further studies are needed to confirm these initial observations and to explore the mechanisms of irisin effects in the reproductive system.

1. Introduction

The infertility rate in the United States is believed to be between 7 and 16% [1], while 2% of reproductive age women have at least one clinical visit for this condition and 10% of women have received infertility services in their lifetime [2]. Reproduction and fertility are closely linked to energy metabolism and the endocrine function of the adipose tissue, as either obesity or insufficient weight are associated with infertility in both male and female. Irisin is a recently discovered myokine and adipokine, a transcriptional activator of nuclear receptor peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) [3]. Irisin synthesis is stimulated by the muscle PGC-1α after exercise or cold exposure, resulting in an increase of uncoupling protein 1 (UCP1) gene expression and oxygen consumption [3]. Irisin levels correlate with adiposity markers, such as body weight, body mass index (BMI), waist circumference and fat mass and are higher in men than in women [4–9]. Elevated irisin levels were found in women with polycystic ovarian syndrome (PCOS), which often presents with obesity, insulin resistance and hyperandrogenism [10]. Interestingly, metformin treatment of PCOS women resulted in lowering of serum irisin concentrations [11].

In this study, we explored whether irisin impacts the hypothalamus-pituitary-gonadal axis by directly affecting pituitary and/or ovarian reproductive functions. We used two *in vitro* systems, a commercially available murine pituitary cell line mPit12 which produces gonadotropins and responds to GnRH stimulation,

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and primary cultures from human ovarian granulosa cells obtained from women undergoing *in vitro* fertilization (IVF) procedure.

2. Materials and methods

2.1. Human subjects

All human research studies were reviewed and approved by the Institutional Review Boards of Beth Israel Medical Center and Weill Medical College of Cornell University in New York City.

2.2. Reagents

GnRH, irisin, and insulin were obtained from Phoenix Pharmaceuticals [Burlingame, CA].

2.3. Cell cultures and treatments

Murine pituitary cell culture

The adult mouse pituitary cell line mPit12 [Cedarline CELLutions Biosystems, Burlington, NC] was cultured according to manufacturer's recommendations. The cells were grown to 70–90% confluence, trypsinized and transferred to 6-well tissue culture plates containing DMEM with 2% FBS for 48 h. The cells were then trypsinized again, resuspended in DMEM medium to 5×10^4 cells/ml, plated into a 24-well tissue culture plates and incubated for additional 24 h. Cells were treated with vehicle (DMSO) or either gonatropin-releasing hormone (GnRH) (10–50 nM) or irisin (25–150 ng/ml) with or without GnRH (25 nM).

Human granulosa cell culture

Human granulosa cells (GCs) were purified from follicular fluid samples obtained during *in vitro* fertilization (IVF) [Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY]. The cells were harvested by centrifugation and resuspended in HBSS medium [Thermo Fisher Scientific, Springfield Township, NJ]. The GCs were then purified twice on Percoll gradient and cultured as previously described [12] in medium M199 [Sigma Aldrich, St. Louis, MO] supplemented with 10% FBS for 48 h, followed by incubation with 2% FBS medium for additional 24 h.

2.4. Hormone measurements

LH and estradiol (E2) concentrations in the conditioned cell culture medium were measured using ELISA [Phoenix Pharmaceuticals, Burlingame, CA]. The minimal detectable concentrations of these assays were 1 mIU/ml for LH and 10 pg/ml for E2.

2.5. Statistical analyses

One- or two-way analysis of variance (ANOVA) was used to evaluate the effect of irisin (with or without another hormone) on the levels of LH or E2. Pairwise multiple comparisons were carried out using the Tukey-Kramer method. A difference between the experimental conditions and control was considered statistically significant if $p < 0.05$.

3. Results and discussion

To test our *in vitro* system, we treated mPitA12 cell for 24 h with increasing concentrations of GnRH (ranging from 10 to 50 nM) and measured LH production using ELISA. As expected, GnRH treatment produced significant dose-dependent stimulatory effect on LH ($p = 0.0005$) across the GnRH concentrations (Fig. 1A), thus validating this model.

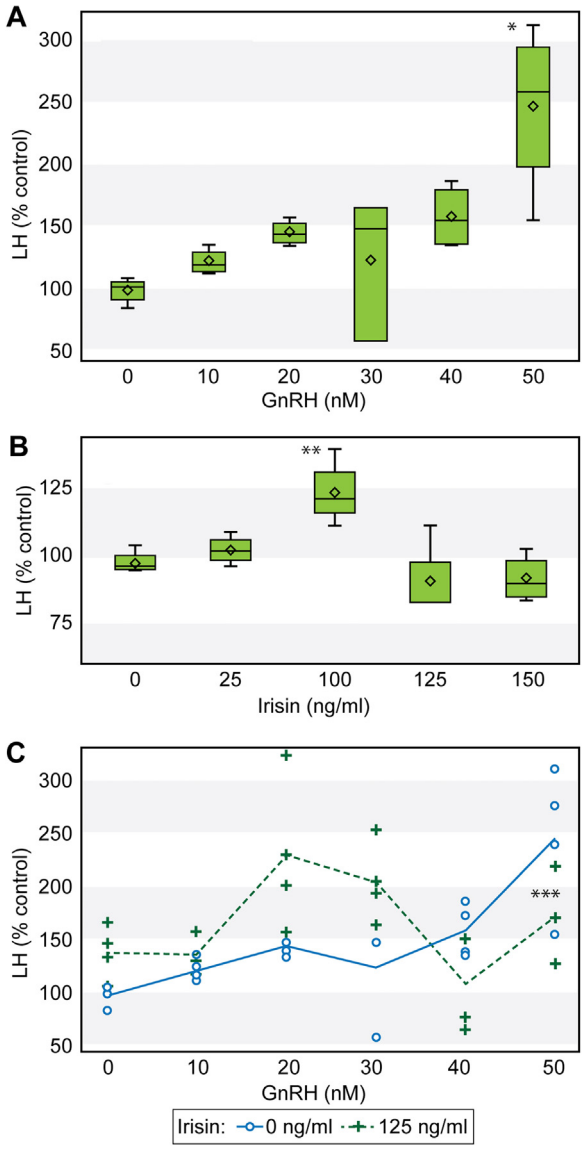

Fig. 1. Effect of GnRH or irisin on LH production in mPitA12 cells. Cells were incubated with either vehicle or increasing concentrations of GnRH (10–50 nM) (A), irisin (25–150 ng/ml) (B) or combination of both (C). LH concentration in the conditioned culture medium was measured by ELISA. Data are presented as % of control. * $p < 0.0005$ for the dose-dependent effect; ** $p < 0.0015$ for the specific concentration of irisin (100 ng/ml); *** $p < 0.0019$ for the difference in pattern with and without irisin.

Further, we investigated the effect of irisin on LH secretion using concentrations of irisin ranging from 25 to 150 ng/ml for 24 h. We observed a significant effect of irisin on LH production ($p = 0.0015$, Fig. 1B), with LH levels at irisin concentration of 100 ng/ml being significantly greater than at other irisin concentrations. At all other concentrations tested, irisin-stimulated LH levels did not differ from control.

When the cells were cultured with a combination of irisin (125 ng/ml) and GnRH (0–50 ng/ml), the stimulatory effect of GnRH on LH production exhibited two different patterns for absence of irisin *vs.* presence of irisin ($p = 0.0019$) (Fig. 1C). In the absence of irisin, there was a general upward trend in LH as the concentration of GnRH increased. However, in the presence of 125 ng/ml of irisin, there was no consistent dose-response relationship between GnRH and LH.

For the next group of experiments we used human GC harvested from follicular fluid obtained from patients undergoing