Update: Does Irisin Have an Effect on Female Reproductive Function? Initial In-Vitro Studies

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Abstract

Irisin is a recently discovered hormone which is secreted by muscle and adipose tissue and plays a role in fat and energy metabolism. Because energy metabolism is closely linked to reproduction, we hypothesized that irisin may have effects in the reproductive system. In this study we examined the effects of irisin on gonadotropin (LH and FSH) secretion in murine pituitary cells and on estradiol (E2) secretion in human granulosa cells.

Methods: The adult mouse pituitary cell lines (mPitA12 and mPitA19, CELLutions Biosystems Inc. and UCLA at San Diego, respectively) were incubated with or without increasing concentrations of irisin (0-150ng/mL) and/or GnRH (0-50nM).

Human granulosa cells from in-vitro fertilization were cultured with and without irisin (0-150ng/mL) and/or insulin (0-10,000ng/mL).

ELISA was used to measure LH/FSH or E2 concentrations in the conditioned culture medium. Two-way analysis of variance (ANOVA) was used to compare mean LH/FSH or E2 for various combinations of GnRH and/or irisin or insulin and/or irisin.

Results: In the pituitary cell experiments, GnRH alone (without irisin) stimulated LH secretion in a dose-dependent manner ($p<0.0005$), thus validating the assay. Irisin alone (without GnRH) did not produce significant stimulation of LH or FSH production. When irisin and GnRH were used together, the stimulatory effect of GnRH on LH production was abolished.

In the granulosa cell experiments, E2 concentration in the medium increased as insulin concentration increased ($p< 0.0494$), thus validating the assay. Irisin alone (without insulin) stimulated production of E2 ($p< 0.0002$). With both irisin and insulin in the system, there was significant irisin/insulin interaction with stimulating effects of either irisin or insulin on E2 production abolished.

Conclusions: In these preliminary in-vitro experiments, irisin appeared to have effects in both pituitary and ovarian cells, suggesting that irisin may play a role in regulating reproductive function. To confirm and to understand the mechanisms of these interactions will require further study.

Introduction

Irisin is a recently discovered hormone secreted by muscle and adipose tissue (1). Irisin is responsible for "browning" of fat and therefore is implicated in playing a role in fat and energy metabolism, insulin resistance and metabolic syndrome (MetS). Irisin is cleaved from Fibronectin type III domain-containing protein 5 (FNDC-5)(2) and is induced by PGC1α, a PPARγ co-activator – a modulator of uncoupling protein-1 responsible for thermogenesis in brown adipose tissue (BAT). Circulating irisin concentrations correlate positively with BMI (3,4). Because energy, metabolism and reproduction are closely linked, we examined the effects of irisin on LH or FSH secretion in murine pituitary cells and on estradiol (E2) secretion in human granulosa cells.

Methods

The adult mouse pituitary cell lines (mPitA12, mPitA19) were obtained from CELLutions Biosys(mPitA12 or mPitA19) were obtained from CELLutions Biosystems Inc. or Cedars Sinai Medical Center. Pituitary cells were incubated in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 UI penicillin and 100 mg/ml streptomycin at 37°C with 5% CO_2 grown to 70-90% confluence, trypsinized and then incubated for 48 hours under the same conditions. The cells were further incubated for another 24 hours in 6-well plates with 2% fetal bovine serum (FBS), 100 UI penicillin and 100 mg/ml streptomycin at 37°C with 5% CO_2. Cells were incubated for 19 hours with the same medium containing either GnRH (0, 10, 20, 30, 40, 50 nM), irisin alone (0, 25, 100, 125, 150 ng/ml), or irisin (0 or 125 ng/ml) plus GnRH (10, 20, 30, 40, 50 nM). LH or FSH concentration was measured by ELISA (MyBioSource San Diego, CA).

Human granulosa cells were obtained from the IVF program at NY-Presbyterian hospital-Weill Cornell. They were purified on the Percoll gradients (40% Percoll/Hank's balanced salt solution) as previously described. Purified human granulosa cells were counted using hemocytometer, and 1mL of 0.5 × 10^5cells/mL suspension was placed in 24 well tissue culture plates (5). The cells were cultured for 48 hours at 37°C, 5% CO_2, 90% humidity in M199 medium supplemented with 10% FBS, 10 µg/mL gentamicin, and 250 ng/mL amphotericin B. After 48 hours of incubation, the medium supplemented with 10% FBS was replaced by a medium with 2% FBS, in which the cells were incubated for additional 24h before 10µM testosterone (substrate) was added to the same medium. Cells were incubated with either insulin alone (0, 10, 100, 1,000, 10,000 ng/ml), irisin alone (0, 25, 75, 100, 125, 150 ng/ml), or insulin (0, 50 ng/mL) plus irisin (0-150 ng/mL). E2 concentration was measured by ELISA (ALPCO Salem, NH).

Statistics: Two-way analysis of variance (ANOVA) was used to compare mean LH, FSH or E2 for various combinations of GnRH and irisin or insulin and irisin.

Results

Figure 1. GnRH effect on LH production in mouse pituitary cells

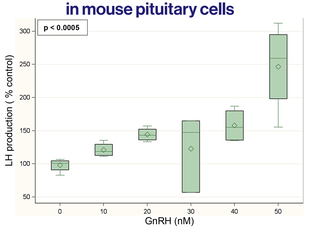


Figure 2. LH production in response to irisin alone.

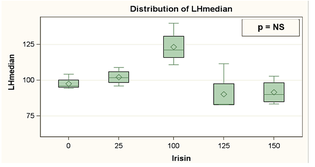


Figure 3. Irisin + GnRH interaction; effect on LH production.

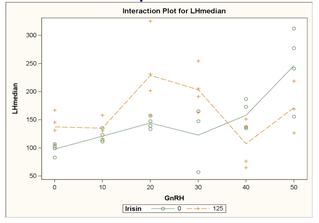


Figure 4. FSH production with irisin alone

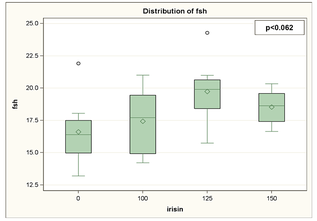


Figure 5. Effect of insulin on E2 production in human granulosa cells

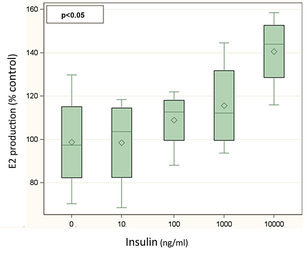


Figure 6. Irisin effect on E2 production in human granulosa cells

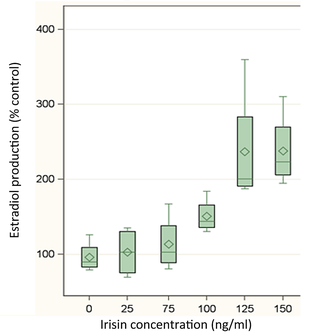
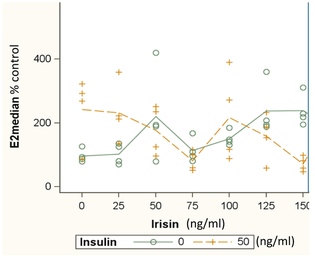


Figure 7. Interaction of irisin and insulin; effect on E2 production



In the pituitary cell experiments, GnRH alone stimulated LH secretion in a dose-dependent manner ($p<0.0005$), thus validating the assay. Irisin alone had no effect on either LH or FSH secretion. When irisin was combined with GnRH, stimulating effect of GnRH or LH production was abolished.

In granulosa cells, E2 concentration in the medium increased as insulin concentration increased ($p<0.0494$), thus validating the assay. Irisin alone stimulated production of E2 in a dose-dependent manner ($p< 0.0002$). In the presence of both irisin and insulin, stimulatory effect on E2 production previously seen with each of these hormones was not observed.

Conclusion

In these preliminary in-vitro studies, irisin was not found to have independent effects on gonadotropin production. When GnRH and irisin were used in combination, however, stimulatory effect of GnRH on LH production was abolished.

Irisin alone or insulin alone stimulated E2 production in human granulosa cells. When a combination of irisin and insulin was used, no stimulating effect on E2 production was observed.

Taken together, these preliminary experiments suggest that irisin may have effects on reproductive function. To confirm these data and to explore the mechanisms of these effects will require future studies.

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