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Mitochondrial Fusion Promotes Steroidogenesis in MA10 Leydig Cells

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Summer Undergraduate Research Program

Introduction

Infertility affects 1 out of every 6 couples, worldwide, with male infertility playing a primary factor in a third of all cases. Dysregulation of sex hormones is a major cause of infertility. Male hypogonadism is a condition in which the testis does not produce adequate concentrations of testosterone. Males suffering from hypogonadism can be born with the condition or develop it later in life, often from acute injury or infection. Male hypogonadism is treated with testosterone replacement to return testosterone levels to normal. However, one side effect of testosterone treatment is infertility. Understanding mechanisms that regulate the synthesis of sex steroids holds great potential to positively impact reproductive health and overall quality of life. Mitochondria play a key role in the synthesis of all steroid hormones. The first and rate-limiting step in the production of all steroid hormones is the transfer of cholesterol from the outer mitochondrial membrane to the inner membrane. In the current study, we examined the hypothesis that mitochondrial fusion promotes acute steroid synthesis in LH-responsive Leydig cells.

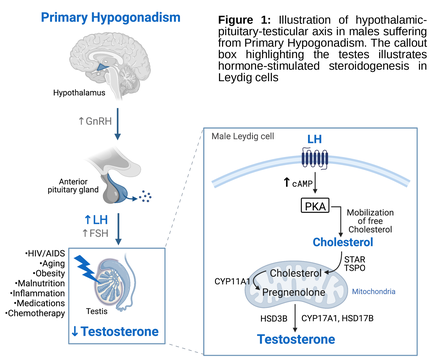
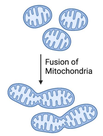


Figure 1: Illustration of hypothalamic-pituitary-testicular axis in males suffering from Primary Hypogonadism. The callout box highlighting the testes illustrates hormone-stimulated steroidogenesis in Leydig cells

Hypothesis

In Experiment 1, we hypothesize that mitochondrial fusion is essential for acute LH-induced steroid production and disruption of mitochondrial fusion leads to attenuated steroidogenesis in MA10 Leydig cells.

In Experiment 2, we hypothesized that the administration of commercially available compounds known to promote the elongation of mitochondria promotes increased sex steroid synthesis following acute LH-induced steroid production in MA10 Leydig cells.



Experimental Design

- The Mouse tumor Leydig cell line (MA10) was used as an *in vitro* model for understanding the mechanisms regulating Leydig cell steroidogenesis.
- Live-cell imaging using high-resolution confocal microscopy was performed to visualize mitochondrial morphology.
- Progesterone concentrations from the conditioned medium were determined using a commercially available progesterone ELISA.
- Western blotting was used to determine protein expression of mitochondrial fusion proteins, MFN2 and OPA1.

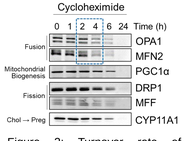


Figure 2: Turnover rate of proteins involved in the regulation of mitochondrial dynamics

Results

Experiment 1.1: Luteinizing hormone stimulates the fusion of mitochondria

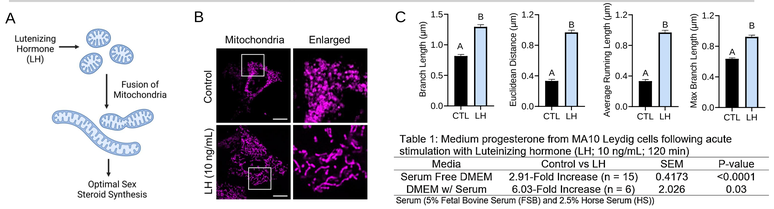


Table 1: Medium progesterone from MA10 Leydig cells following acute stimulation with Luteinizing hormone (LH; 10 ng/mL; 120 min)

Media	Control vs LH	SEM	P-value
Serum Free DMEM	2.91-Fold Increase (n = 15)	0.4173	<0.0001
DMEM w/ Serum	6.03-Fold Increase (n = 6)	2.026	0.03

Serum (5% Fetal Bovine Serum (FSB) and 2.5% Horse Serum (HS))

Figure 3: The effects of Luteinizing hormone (LH) on mitochondrial morphology. Panel A: Illustration of the effects of LH on mitochondrial morphology in MA10 Leydig cells. Panel B: Representative confocal micrograph obtained from MA10 cells treated with control or LH (10 ng/mL) for 120 min. Panel C: Quantification of mitochondria length. Micron bar represents 10 μM. Bars with different letters^{AB} differ significantly compared to control ($P < 0.05$).

Results

Experiment 1.2: Inhibition of mitochondria fusion attenuates LH-stimulated progesterone synthesis

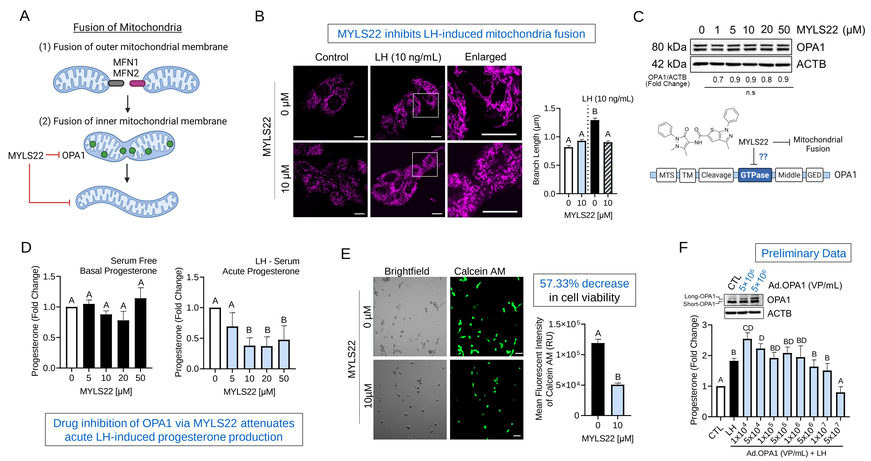


Figure 4: The effects of MYLS22 on mitochondrial morphology and LH-induced steroidogenesis in MA10 Leydig cells. Panel A: Illustration of mitochondrial fusion and MYLS22 inhibition of OPA1. Panel B: Representative micrograph of mitochondria obtained from MA10 Leydig cells pretreated with or without MYLS22 (10 μM) for 60 min and subsequently stimulated with LH (10 ng/mL) for 120 mins. From left to right: Control, LH, and an enlarged image of LH. From top to bottom: 0 μM and 10 μM MYLS22. Panel C: Representative Western blot of OPA1 expression following treatment with MYLS22 (0-50 μM) for 3 hours. Illustration of MYLS22 possible mechanism of OPA1 inhibition. Panel D: Progesterone production obtained from MA10 Leydig cells pretreated with or without MYLS22 (0-50 μM) for 60 min and subsequently stimulated with LH (10 ng/mL) for 120 mins. Panel E: Representative micrograph and analysis of Calcein AM staining (assessment of viability) obtained from MA10 Leydig cells pretreated with or without MYLS22 (10 μM) for 3 hours. Panel F: Progesterone production obtained from MA10 Leydig cells treated with increasing concentrations of replication-deficient adenoviruses (Ad) expressing OPA1. Representative Western blot obtained from MA10 Leydig cells treated with $1x10^4$ and $5x10^4$ VP/mL Ad.OPA1. Control represents Ad.GFP ($5x10^5$ VP/mL). Micron bar represents 10 μM. Bars with different letters^{ABCD} differ significantly compared to control ($P < 0.05$).

Experiment 2.1: Effects of Mitochondrial fusion promoter 1 on LH-stimulated progesterone synthesis

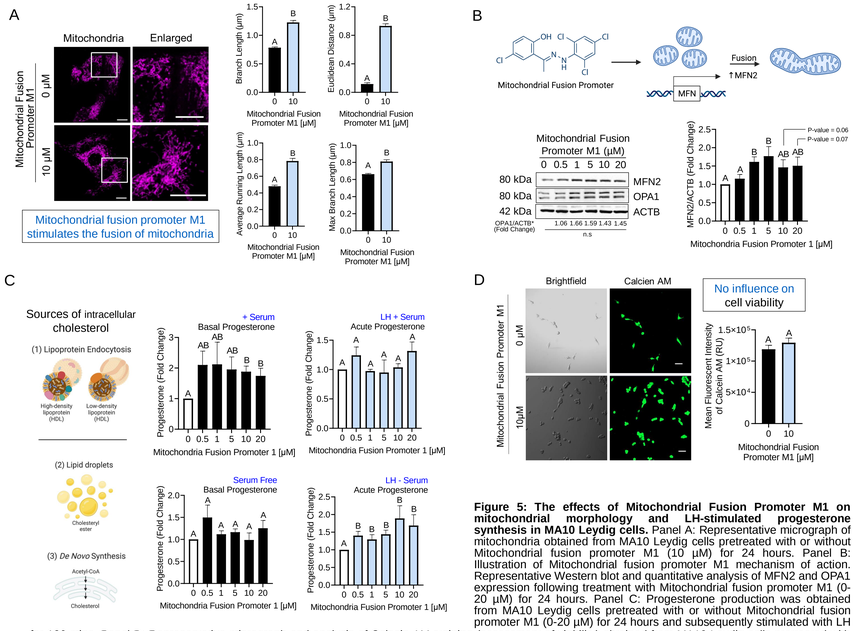


Figure 5: The effects of Mitochondrial Fusion Promoter M1 on mitochondrial morphology and LH-stimulated progesterone synthesis in MA10 Leydig cells. Panel A: Representative micrograph of mitochondria obtained from MA10 Leydig cells pretreated with or without Mitochondrial fusion promoter M1 (10 μM) for 24 hours. Panel B: Illustration of Mitochondrial fusion promoter M1 mechanism of action. Representative Western blot and quantitative analysis of MFN2 and OPA1 expression following treatment with Mitochondrial fusion promoter M1 (0-20 μM) for 24 hours. Panel C: Progesterone production was obtained from MA10 Leydig cells pretreated with or without Mitochondrial fusion promoter M1 (0-20 μM) for 24 hours and subsequently stimulated with LH for 120 mins. Panel D: Representative micrograph and analysis of Calcein AM staining (assessment of viability) obtained from MA10 Leydig cells pretreated with Mitochondrial fusion promoter M1 (10 μM) for 24 hours. Micron bar represents 10 μM. Bars with different letters^{AB} differ significantly compared to control ($P < 0.05$).

Results

Experiment 2.2: Effects of Leflunomide on LH-stimulated progesterone synthesis

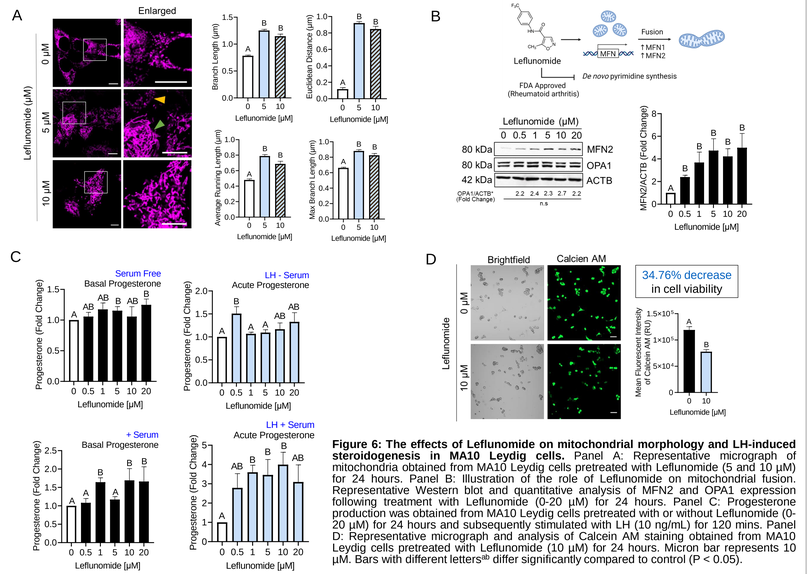


Figure 6: The effects of Leflunomide on mitochondrial morphology and LH-induced steroidogenesis in MA10 Leydig cells. Panel A: Representative micrograph of mitochondria obtained from MA10 Leydig cells pretreated with Leflunomide (5 and 10 μM) for 24 hours. Panel B: Illustration of the role of Leflunomide on mitochondrial fusion. Representative Western blot and quantitative analysis of MFN2 and OPA1 expression following treatment with Leflunomide (0-20 μM) for 24 hours. Panel C: Progesterone production was obtained from MA10 Leydig cells pretreated with or without Leflunomide (0-20 μM) for 24 hours and subsequently stimulated with LH (10 ng/mL) for 120 mins. Panel D: Representative micrograph and analysis of Calcein AM staining obtained from MA10 Leydig cells pretreated with Leflunomide (10 μM) for 24 hours. Micron bar represents 10 μM. Bars with different letters^{AB} differ significantly compared to control ($P < 0.05$).

Conclusions

- LH stimulates the fusion of mitochondria in MA10 cells.
- Drug inhibition of mitochondrial fusion attenuates LH-induced steroid synthesis
- Supplementing cells with pharmaceutical compounds known to promote the fusion of mitochondria may serve beneficial for increasing endogenous steroid synthesis in MA10 Leydig cells.

Taken together, these findings place mitochondrial morphology as an important target downstream of LH for the synthesis of steroid hormones

Future Directions

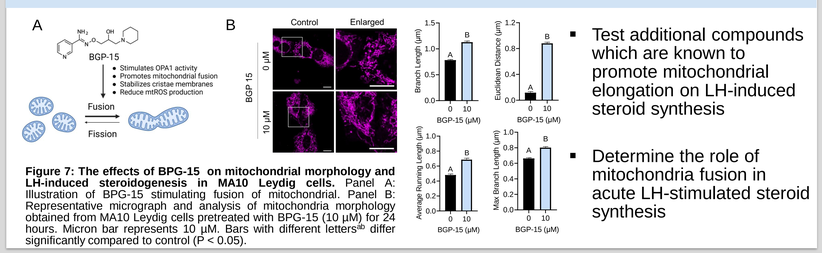


Figure 7: The effects of BPG-15 on mitochondrial morphology and LH-induced steroidogenesis in MA10 Leydig cells. Panel A: Illustration of BPG-15 stimulating fusion of mitochondrial. Panel B: Representative micrograph and analysis of mitochondria morphology obtained from MA10 Leydig cells pretreated with BPG-15 (10 μM) for 24 hours. Micron bar represents 10 μM. Bars with different letters^{AB} differ significantly compared to control ($P < 0.05$).

- Test additional compounds which are known to promote mitochondrial elongation on LH-induced steroid synthesis
- Determine the role of mitochondria fusion in acute LH-stimulated steroid synthesis

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