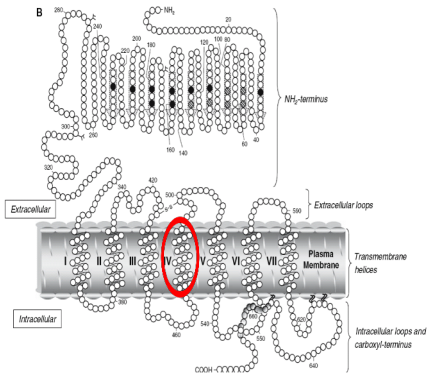
Effects of Caveolin Disruption on signaling from the hFSH receptor

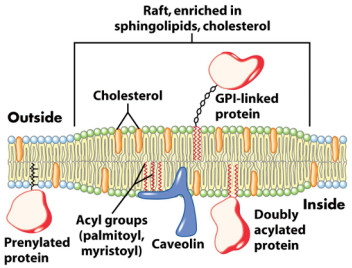
Maggie Weichert 22', Union College Biology Department, Schenectady, NY

Introduction

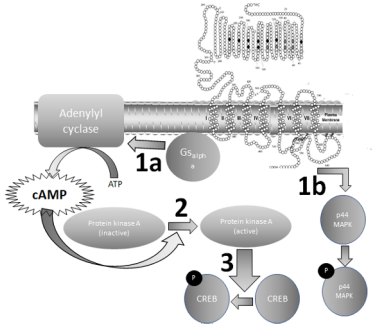
Follicle Stimulating Hormone Receptor and Caveolin



- FSHR is a G-Protein Coupled Receptor (GPCR) activated by FSH
- Located in the membranes of Sertoli Cells in testes and granulosa cells in ovaries
- FSH aids in follicle maturation and estrogen production in women and sperm development in men
- The protein caveolin is thought to interact with transmembrane domain IV (circled) to promote the binding of the receptor to the caveolae, a type of lipid raft
- Lipid rafts support a diverse group of receptors that are needed at the cell membrane



Current FSHR signaling pathway model & cAMP detection



1a. When FSH binds to FSHR the G protein alpha subunit activates adenylyl cyclase to stimulate production of cAMP
1b. FSH activates downstream phosphorylation of p44 MAP Kinase
2. cAMP activates protein kinase A
3. Protein kinase A activates the MAP kinases and the cAMP response element binding protein (CREB)

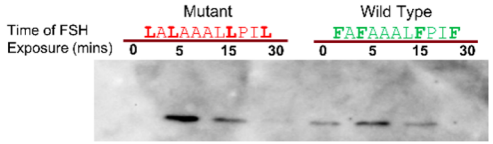
Hypothesis

HEK293-hFSHR cells that are treated with the wild-type (interfering) peptide will express decreased levels of cAMP. There should be no difference in the level of cAMP in the cells treated with the mutant peptide and the cells treated with no peptide.

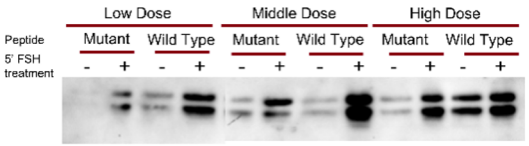
Tat -WT Caveolin binding motif peptide	ygrkkrrqrrrFAFAAALFPIF
Tat -Mutant Caveolin binding motif peptide	ygrkkrrqrrr**L**A**L**AAAL**L**PI**L**

Wild type and mutant caveolin binding motifs. The Tat peptide is in small letters and the hFSHR caveolin binding motif is in capital letters. Mutated residues are in bold.

Other Experiments from the Cohen Lab



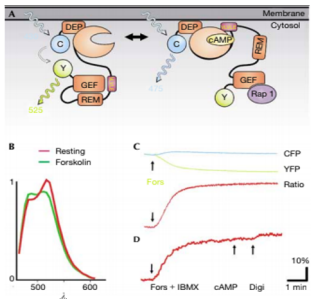
Western blot analysis of p-CREB levels in response to hFSH treatment following pretreatment with different doses of peptide mimetics of the hFSHR-caveolin interaction motif.



Western Blot analysis of p44 levels in response to hFSH treatment for different times following pretreatment with WT and MU peptide mimetics of the hFHSR-caveolin interaction motif.

Experimental Approach

- **CREB and p44 pathways measure hFSHR response indirectly. This experiment attempts to measure hFSHR activation more directly through monitoring cAMP production**



- EPAC serves as a highly sensitive CAMP indicator. When expressed in mammalian cells, EPAC shows significant FRET.
- Fluorescence resonance transfer energy (FRET) is expressed as a ratio of CFP to YFP.
- FRET decreases in response to CAMP production and fully recovers in the presence of CAMP lowering agonists.
- **No cAMP** = Emission at 530 nm
- **CAMP** = Emission changes to 480nm

Prepare 96 well dish of hFSHR-EPAC cells.

↓

Pretreatment
Aspirate wells and wash with S/F medium
Introduce WT and MU peptides into cells
Incubate 1 hour

↓

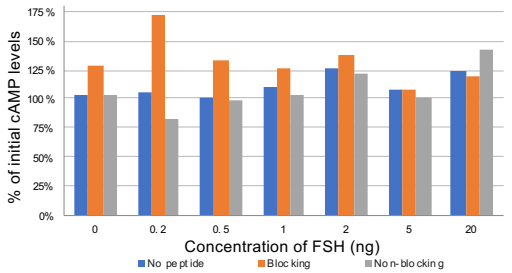
Remove medium and wash with 1X PBS and take baseline reading

↓

Treatment & Analyze
Treat with 1x PBS with IBMX, forskolin, FSH
Take kinetic read for 15 minutes
Take final read

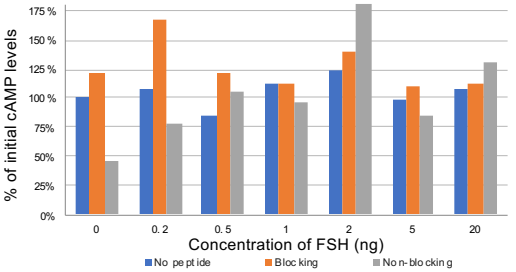
Results

Average Difference in cAMP levels at 5 Minutes



This figure compares the ratio of LM1 (480nm, no FRET) to LM2 (530nm, FRET) at t=0 to the ratio of LM1 to LM2 at t = 5 minutes. The difference in cAMP levels between t=0 and t=5 in each well were then averaged.

Average Difference in cAMP levels at 15 Minutes



This figure compares the ratio of LM1 (480nm, no FRET) to LM2 (530nm, FRET) at t=0 to the ratio of LM1 to LM2 at t = 15 minutes. The difference in cAMP levels between t=0 and t=15 in each well were then averaged.

Conclusions

- In the presence of the wild type (blocking) peptide, there is an increase in basal levels of cAMP, which does not support the original hypothesis. This may suggest that caveolin plays a role in turning the hFSH receptor off.
- This observation is consistent with the western blot analysis from the Cohen lab, but inconsistent with known literature that suggests caveolin blocking peptides have a diminished capacity to stimulate the receptor (Roh *et al,* 2014).
- Although it is unclear what role the mutant (non-blocking) peptide may be playing, our data suggests that the tat mutant peptide sequence may not be the best control.
- In the future, this experiment should be repeated using a mutant peptide with a scrambled caveolin binding motif sequence.

References and Acknowledgements

- Angela Stewart, Brian D. Cohen, Neuroscience Honors Thesis: "Modulating Human Follicle Stimulating Hormone Receptor Action Using Small Molecules."Union College Biology Department, Schenectady, NY.
- Chini, B., and M. Parenti. (2004) "G-protein coupled receptors in lipid rafts and caveolae: how, when and why do they go there?" *Journal of Molecular Endocrinology*.
- Cohen Brian D., and Dias James A. (2019) Follitropin. *Reference Module in Biomedical Sciences.* Elsevier.
- Neal, J. Matthew. (2002) How the Endocrine System Works. Blackwell Science.
- Ponsioen, B. et al. (2004) "Detecting cAMP induced Epac activation by fluorescence resonance energy transfer: Epac as a novel cAMP indicator." *EMBO Reports* (2004) Vol 5, No. 12.
- Roh, *et al.* (2014) "Lipid rafts serve as signaling platforms for mGlu1 receptor mediated calcium signaling in association with caveolin." *Molecular Brain,* 7:9.
- Brian D. Cohen, PhD