Follicle Stimulating Hormone Receptor Resides in Detergent Resistant Membrane Microdomains

By

Johanna Geoghegan

****

Submitted in partial fulfillment
of the requirements for
Honors in the Department of Biological Sciences

UNION COLLEGE
June, 2011

ABSTRACT

GEOGHEGAN, JOHANNA. Follicle Stimulating Hormone Receptor Resides in Detergent Resistant Membrane Microdomains. Department of Biological Sciences, June 2011.

ADVISOR: Professor Brian Cohen

Follicle stimulating hormone (FSH) is an anterior pituitary glycoprotein hormone crucial for proper spermatogenesis in males and oocyte development in females. The follicle stimulating hormone receptor (FSHR) is a G-protein coupled receptor found exclusively on the membranes of Sertoli cells in the testes and granulosa cells in the ovaries. It has been proposed that FSHR localizes to detergent resistant portions of the membranes called lipid rafts, which are membrane microdomains that contain a high percent composition of cholesterol and sphingolipids. To determine if FSHR resides in these membrane microdomains, strong detergents were used to extract and isolate detergent resistant membranes. Prior to extraction, cells were treated with FSH and/or the cholesterol depleting drug methyl- β-cylcodextrin (MβCD), and western blot analysis was conducted. FSHR was observed in both the detergent soluble and insoluble portions of the membrane, suggesting that there are two populations of FSHR, and the receptor is more likely to reside in lipid raft or detergent resistant fractions. FSH treatment resulted in increased FSHR residency in lipid rafts fractions. Understanding FSHR residency in the plasma membrane may lead to greater understanding of its signaling pathways and potential human health implications for infertility treatment and contraception development.

INTRODUCTION

The endocrine system is an intricate communication system between parts of the brain and various target organs involved in reproduction, metabolism, and immune response. Communication is achieved through hormones, chemical messengers which travel to targets through the blood stream. Response to a hormone is determined by the presence of a hormone specific receptor on the target cell, and the resulting signal transduction may cause modification of proteins and upregualtion or inhibition of the expression of specific genes.[1]

Follicle stimulating hormone (FSH) is a hormone involved in the hypothalamic-pituitary-gonadal axis, and it is essential for gonad maturation and gamate formation in males and females. FSH is a member of the glycoprotein hormone family, which also includes thyroid stimulating hormone, chorionic gonadotropin, and luteinizing hormone. Glycoprotein hormones are heterodimers that share a common alpha subunit but contain distinct hormone specific beta subunits.[1,2] FSH is secreted by the gonadotrophs in the anterior pituitary when stimulated by gonadotropin-releasing hormone from the hypothalamus. FSH binds to the follicle stimulating hormone receptor (FSHR) found exclusively anchored in the membranes of granulosa cells in the ovary and Sertoli cells in the testes.[2]

In males, FSH promotes spermatogenesis by stimulating the Sertoli cells to release growth factors that support the developing sperm in the seminiferous tubules. Growth factors promote the maturation of primary spermatocytes to haploid secondary spermatocytes to spermatids and then finally into mature spermatozoa.[1]

In females, FSH stimulates the maturation of primordial follicles to mature vesicular follicles in the ovary by activating more than 100 different target genes involved in cellular proliferation, differentiation, antrum formation and oocyte maturation in the granulosa cell (Figure 1). Follicles contain the oocyte, which is surrounded by the granulosa cells, a basal lamina, and peripheral thecal cells.[3] Granulosa cells, as a result of the maturation of follicles, produce estrogen. Estrogen is involved in expression of secondary sex characteristics and stimulation of luteinizing hormone secretion, which is responsible for the rupturing of the mature vesicular follicles, or ovulation.[3]

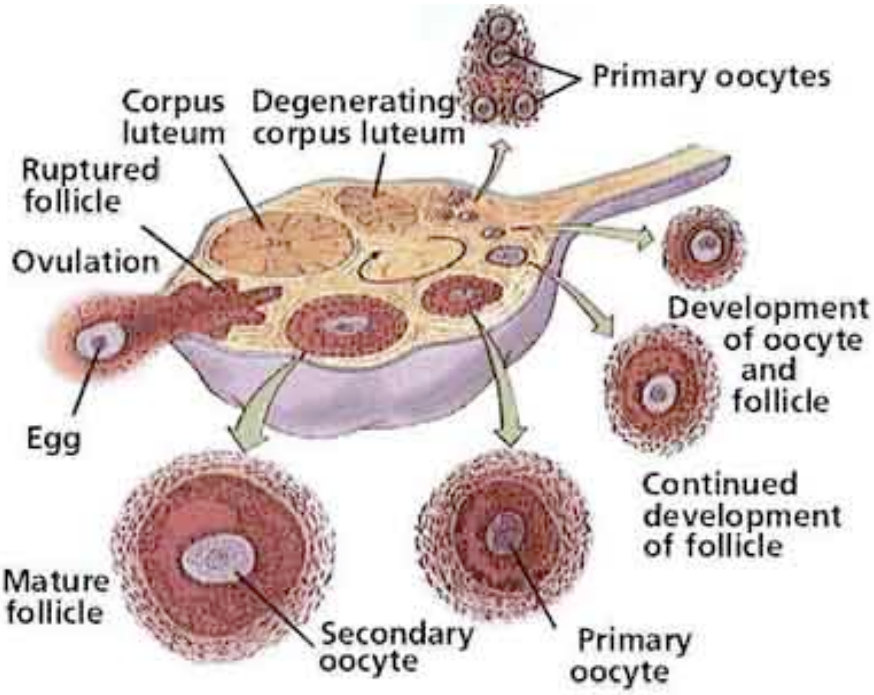

Figure 1: Maturation of ovarian follicles in ovary[4]

The function of a receptor is to relay extracellular stimuli, such as the presence of a hormone, to intracellular effector molecules. The FSHR is a G-protein coupled receptor (GPCR), and is composed of 695 amino acids. The mature glycosylated receptor ranges from 75 to 76.5 kDa.[5] A representaion ot the FSHR can be seen in Figure 2. The

transmembrane domain of the receptor contains seven hydrophobic transmembrane alpha helices connected by alternating extracelluar and intracellular loops, which is typical of GPCRs.[5] Additionally, the FSHR has a putative caveolin interaction motif, ΦXΦXXXXΦXX, in the fourth transmembrane domain that may influence the location of its residency in the plasma membrane.[6] The receptor also contains an extracellular N-terminus, and an intracellular C-terminus.[7] The extracellular domain has notable leucine rich repeats throughout, which are thought to help with interaction between this domain and hormone. There are also four potential N-linked glycosylation sites in the extracellular domain of human FSHR that could be involved in hormone binding or proper protein folding.[5]

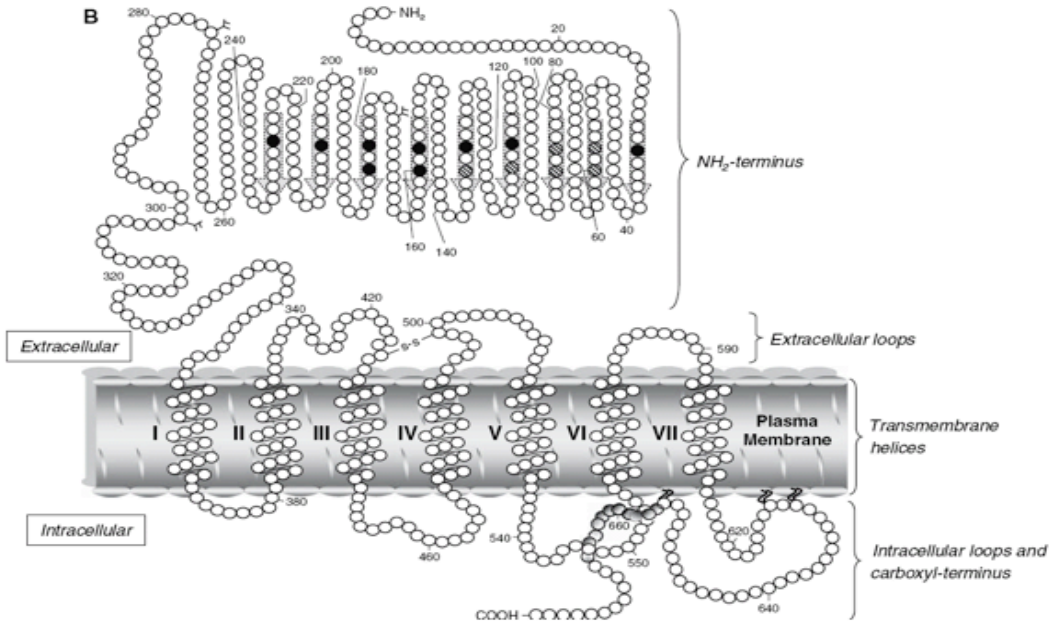

Figure 2: Representation of FSHR displaying the extracellular domain, seven pass transmembrane domain with its alternating intracellular and extracellular loops, and the intracellular domain.[8]

The binding of a ligand to a GPCR induces a conformational change in the receptor that enables it to activate a G-protein. G-proteins are heterotrimeric and

composed of an α, β, and γ subunits. In its inactive state, the α subunit is bound to GDP, but when activated by the receptor, the α subunit binds GTP. Once activated, the α subunit dissociates from the βγ subunit and stimulates downstream effector molecules. The α subunit has intrinsic GTPase activity, and is therefore able to terminate the signal once intracellular signaling cascades have been initiated.[9]

The binding to FSH to its receptor results in the rapid and efficient activation of intracellular signaling cascades that activate various genes in the granulosa and Sertoli cells. The well-characterized signaling cascade activated by the FSHR involves adenylyl cyclase and the associated G-protein, $G_{\alpha}s$.[10] Adenylyl cyclase stimulates cAMP, which ultimately activates cAMP-dependent protein kinase or PKA. PKA phosphorylates transcription factors that regulate gene expression (Figure 3).[9]

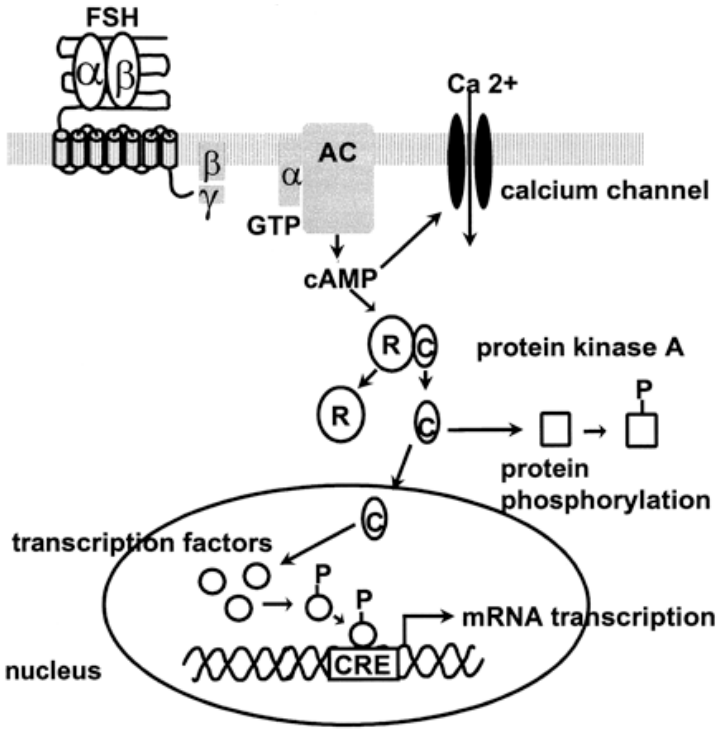

Figure 3: Signal transduction pathway activated by FSH that involves activation of G_s and ultimately PKA [4]

However, FSHR is also capable of activating other signaling cascades such as the EPAC/RAP1 and SRC/RAS/EGFR/MAPK kinase pathways.[10] Furthermore, the PKA pathway generally associated with FSH can also activate multiple additional downstream molecules such as p38MAPK and phosphatidylinositol-3 kinase.[3]

Although GPCRs are able to efficiently activate multiple signaling cascades, G-proteins and associated enzymes are present at low concentrations in mammalian cell membranes. A potential explanation for this discrepancy is receptor localization or residency in membrane microdomains such as lipid rafts. Lipid rafts are areas of the cell membrane that display less fluidity and increased resistance to non-ionic detergents due to the high percent composition of cholesterol and sphingolipids such as sphingomyelin.[11]

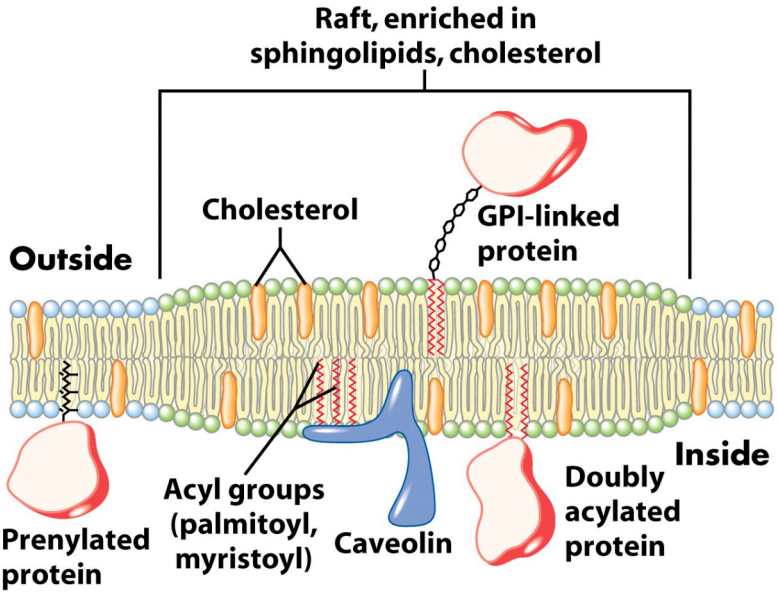

Figure 4: Visual of lipid raft in plasma membrane. Lipid rafts are enriched for cholesterol, sphingolipids, and signaling molecules.[14]

Lipid rafts are enriched for signaling molecules such as G-proteins, GPI-anchored proteins, and adenylyl cyclase.[12] A depiction of the components of lipid rafts can be found in Figure 4. Lipid rafts may facilitate signal transduction by both providing an

arrangement in which signaling components are present in increased concentrations at the site of a potentially active receptor and excluding proteins that could potentially interfere with signal transduction, such as membrane phosphatases.[13] Thus, lipid rafts may function as "signaling platforms."[12]

Previous research in the Cohen lab suggested that disrupting lipid rafts interferes with the receptor's ability to activate downstream signaling components, specifically adenylyl cyclase and the production of cAMP. Lipid rafts were disrupted with the cholesterol depleting drug methyl- β-cylcodextrin (MβCD). While some proteins are confined to membrane microdomains, MβCD treatment disrupts lipid rafts in live cells such that proteins are no longer confined.[12] Figure 5 shows that treatment with MβCD is associated with a decrease in cAMP production, one of FSHR's downstream signaling molecules. Thus, creating cells with effectively no lipid rafts by depleting cholesterol with MβCD interfered with the FSHR ability to activate cAMP.

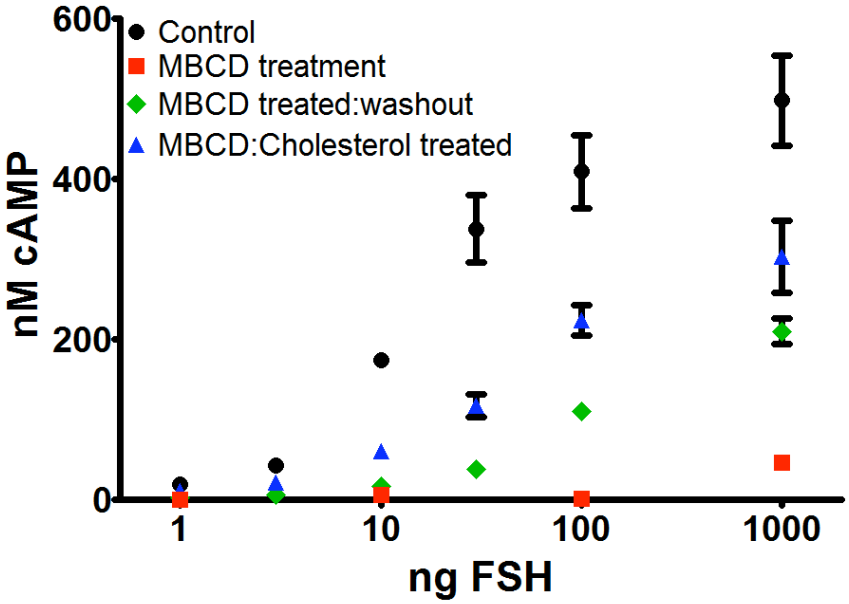

Figure 5: Cholesterol removal with MβCD abrogated cAMP production by HEK293-hFSHR cells. MβCD treatment reduced cAMP production significantly at hFSH doses of 10ng, 100ng, and 1000ng. Removal of MβCD after the 1 hour pretreatment allowed the cells to partially recover, as observed in the difference between treated and cells with the

drug removed (washout) at 100ng and 1000ng hFSH. Each point represents triplicate treatments measured in triplicate.[15]

FSHR may associate with a specific kind of lipid raft, known as caveolae. Caveolae are similar in composition to lipid rafts but are further characterized by the presence of the protein caveolin.[13] Caveolin is a hair-pin like protein responsible for the unique shape of the caveolae, which is often described as a cave-like invagination in the cell membrane that is approximately 50 to 100 nm.[6] A illustration of caveolae, including a morphological comparison to lipid rafts, is shown in Figure 6. Caveolae are found in many types of cells and are involved in phagocytosis, endocytosis, transcellular movement, and cell signaling.[16] Insel et al. found that GPCR and associated signaling components localize to caveolae and that caveolae disruption resulted in altered GPCR signaling.[11]

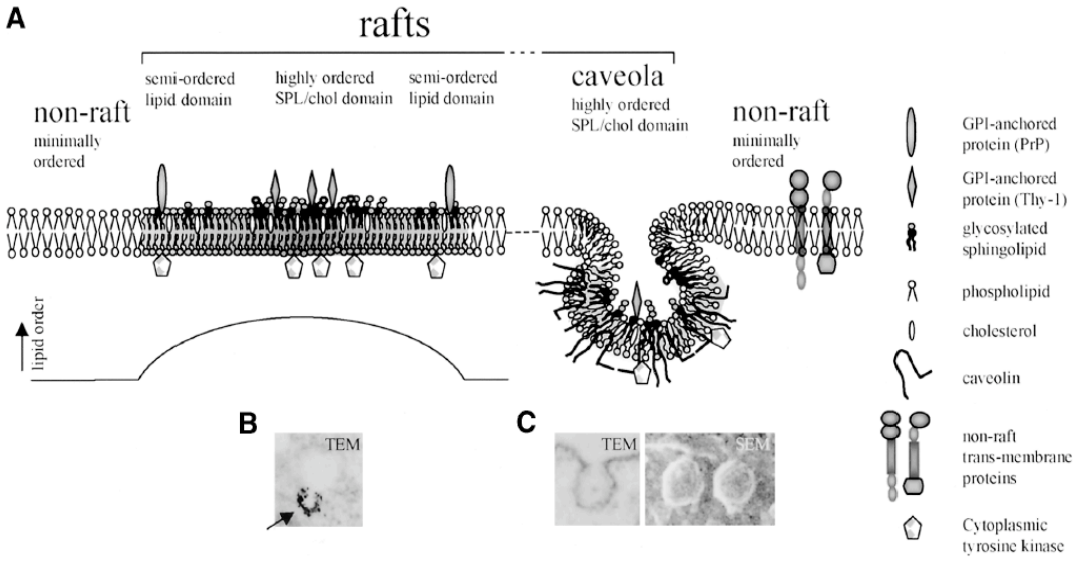

Figure 6: Caveolin causes the characteristic flask shape of caveolae [17]

Further evidence that FSHR may reside in membrane microdomains such as lipid rafts or caveolae includes a putative caveolin interaction motif found in the transmembrane domain of the receptor. A caveolien interaction motif is a conserved amino acid sequence that causes proteins to localize to caveolae. There are three known caveolin interaction motifs, ΦXΦXXXXΦ, ΦXXXXΦXXΦ, and ΦXΦXXXXΦXX, where Φ represents Trp, Phe, or Tyr, and X represents any amino acid.[6] FSHR contains the last caveolin interaction motif in the 4th intracellular transmembrane domain with the amino acid sequence FAFAAALFPIF. This sequence is highly conserved among FSHR amino acid sequences in pigs, cows, sheep, mice, and humans (Figure 7). Conservation of this motif across species suggests that it may be important for receptor function.

Figure 7: Caveolin Interaction Motif found in the fourth transmembrane domain in FSHR. The large letters represent amino acids that were conserved across pigs, cows, sheep, mice, and humans. The smaller letters show where there is some discrepancy in amino acid sequence. The caveolin interaction motif is highly conserved.[17]

Other GPCRs are known to reside in or localize to lipid rafts when bound by an agonist include the angiotensin II (AT-1), serotonin (5HT), gonadotrophin releasing hormone, and oxytocin receptors.[13] Smith and Roess et al. have demonstrated that the luteinizing hormone receptor (LHR), a hormone receptor closely related to the FSHR, also localizes to lipid rafts when stimulated by chorionic gonadotropin hormone. They also found that disruption of lipid rafts with MβCD reduced cell signaling through cAMP.

However, they did not find that disruption of caveolae resulted in altered cell signaling through the LHR.[12] It is interesting to note at this point that the luteinizing hormone receptor only contains a partial putative caveolin interaction motif in the second transmembrane domain. Furthermore, Smith and Roess found that the location of the receptor was dependent on the presence of hormone. Cell membrane fractions were obtained through sucrose gradient fractionation, and when cells were treated with chorionic gonadotropin hormone, functional LHR localized to low density, detergent insoluble membrane fractions with properties consistent with lipid rafts. Without hormone treatment, LHR was found in the bulk membrane.[13]

To characterize the residency of FSHR in the cell membrane, portions of cell membranes of HEK-293 cells that express human FSHR were extracted using detergents of varying strengths. Detergents were used to isolate detergent resistant fractions, or microdomains such as lipid rafts and caveolae, and detergent susceptible fractions. The resulting cell membrane fractions were subjected to SDS PAGE and western blot analysis in which antibodies specific to FSHR were employed to identify the receptor's location. In additional experiments, HEK-293 hFSHR cells were treated with MβCD to determine if lipid raft and caveolae disruption influenced receptor residency and FSH to determine if hormone treatment influences the receptor's location. Understanding the FSHR and its ability to transduce a hormone signal, which may be connected to receptor localization to lipid rafts or caveolae, may provide insight for potential pharmaceutical interventions for infertility treatments and alternative forms of contraception.

METHODS

Cell Culture

Human embryonic kidney (HEK 293) cells were stably transfected with human FSHR and incubated in filtered Dulbecco's Modified Eagle's Medium (DMEM) containing fetal bovine serum and penicillin and streptomycin solution.[18]

Membrane Extraction

Cell culture media was aspirated from four 75cm^2 cell culture flasks at 100% confluency. Five ml of serum free DMEM media was added to one flask, and 5 ml of serum free DMEM media with 2 μl of human FSH was added to the other flask. The flasks were incubated at 37°C for 1 hour. In experiments that included treatment with M*β*CD, four flasks at 100% confluency were used. Five ml of serum free DMEM media were added to two flasks, and 5 ml of serum free DMEM media with 0.066 grams of M*β*CD was added to the other two flasks. The four flasks were incubated at 37°C for 1 hour. Two μl of human FSH were then added to one flask treated with MβCD and one flask that was not treated with MβCD. All flasks were subsequently incubated for 30 min at 37°C.

All media was then aspirated from the flasks and washed gently with 5 ml of 1xphosphate buffered saline (PBS). The 1x PBS solution was then aspirated, and 5 ml of 1XPBS with 5mM of EDTA was added to each flask and allowed to incubate for 5 minutes. The cell suspension was then transferred into 15ml conical tubes and centrifuged at 3000RPMs for 10 minutes.

Pellets were resuspended with 1 ml of 1XMES buffered saline (MBS) and 1% triton with protease inhibitors. Samples were then vortexed well, transferred to dounce homogenizers, and dounced for 10 strokes. Afterwards, samples were transferred to microfuge tubes and centrifuged at 15,000g at 4°C 10 minutes. The supernatant represented membrane proteins extracted with triton, or detergent soluble membrane extracts. The pellet was resuspended with 1 ml 1xMBS and 2% sodium dodecyl sulfate (SDS). Samples were then vortexed and transferred to dounce homogenizers and dounced 10 times. The SDS extracts, or detergent resistant membrane extracts, were centrifuged once more and sonicated.

SDS-PAGE Electrophoresis

A 7.5% resolving gel mixture was made that contained 7.5 ml of water, 3.75 ml of 30% acrylamide and 0.8% bis-acrylamide, 3.75 ml 4x running buffer, 150 μl of 10% SDS, 50μl of 10% APS, and 10μl of TEMED. The resolving gel polymerized for approximately 50 minutes with isopropanol layered on top. After the resolving gel polymerized, the isopropanol was removed, and the 4% stacking gel was added. The stacking gel contained 6.1 ml of water, 1.3 ml of 30% acrylamide and 0.8% bis-acrylamide, 2.5 ml of 4x stacking buffer, 100μ1 of 10% SDS, and 20μl of TEMED. Before the stacking gel polymerized, a 10 well comb was added, and the gel polymerized in approximately 40 minutes.

Protein concentrations were determined by BCA Protein Assays Kit (Pierce) so that a uniform amount of protein was loaded into each lane of the electrophoresis gel. Samples were heated at 100°C for 5 minutes before conducting electrophoresis. A

prestained SDS-Page broad range molecular weight standard was used (either Prosieve color protein marker or Color Plus prestained broad range protein marker from New England Biolabs). Electrophoresis was performed for approximately 30 minutes at 100 volts until the samples migrated through the stacking gel. For the remainder of the time, electrophoresis was conducted at 150 volts.

Gel Transfer

The gels were incubated in transfer buffer for 5 minutes and the membrane was dipped in methanol for 10 seconds and then incubated in transfer buffer for 5 minutes. The samples were transferred from the gel to a 3 ¼" x 2" polyvinyl difluoride (PVDF) membrane using a semi-dry transfer cell (Bio-Rad). The electrophoresis gel was placed on top of the membrane and three pieces of 3 ¼" x 2" Whatman paper were placed on either side. The transfer process was conducted for 15 minutes at 15 volts.

Immunoblotting/Western Blot Analysis

Blots were blocked using 20 ml of milk (5 grams of powdered milk in 100 ml of 1xTBST) overnight at 4°C on a rocker. Primary antibody was then added (5µl in 10 ml of milk) and allowed to incubate for an hour at room temperature. Monoclonal primary antibodies to FSHR were used for one blot and monoclonal primary antibodies to cadherin, gamma-tubulin, or Flotillin-2 were used for the other blot. (Anti-FSHR antibody from James A. Dias Lab, Wadsworth Center, State University of New York at Albany; Anti-cadherin antibody from Sigma; Anti-Flotillin-2 antibody from Cell Signaling) The blots were then washed with 50 ml of 1xTBST three times for five

minutes each wash. The blots were incubated in secondary antibody (2 μl in 10 ml of milk) for 1 hour at room temperature on a rocker. HRP goat anti mouse secondary antibody was used for blots probed with FSHR primary antibody, blots probed with gamma-tubulin primary antibody, and blots probed with cadherin primary antibody (Jackson Immunological Laboratories). Blots probed for flotillin-2 were incubated in primary antibody overnight at 4°C on a rocker. The blots were then washed with 1xTBST, incubated in secondary antibody, washed with 1xTBST, and developed as described above. Goat anti rabbit secondary antibody was used for blots probed with Flotillin-2 primary antibody (Invitrogen). Blots were then washed three times with 50ml of 1xTBST for five minutes for each wash. After the final wash, the blots were developed using super signal west pico chemiluminescent substrate (Thermo Scientific). Each blot was covered with 2 ml of the peroxide solution and 2 ml of enhancer solution (combined just before application) for five minutes. Images were obtained using a FujiImager on chemiluminescent setting.

RESULTS

FSHR found in 2% SDS membrane fractions, primarily in the presence of FSH

The results of the membrane extraction process, SDS PAGE and western blot analysis probed for FSHR can be seen in Figure 8. FSHR has a molecular weight of 79 kDa, but the higher molecular weight observed may be due different glycosylation patterns of the receptor. The higher than expected molecular weight is consistent with the findings of other researchers.[5] The receptor is found primarily in the cellular membrane fractions extracted with 2% SDS, although less distinct bands are present in the 1% triton

membrane fractions as well. Additionally, the FSHR is more likely to be found in the 2% SDS membrane fractions in the presence of FSH.

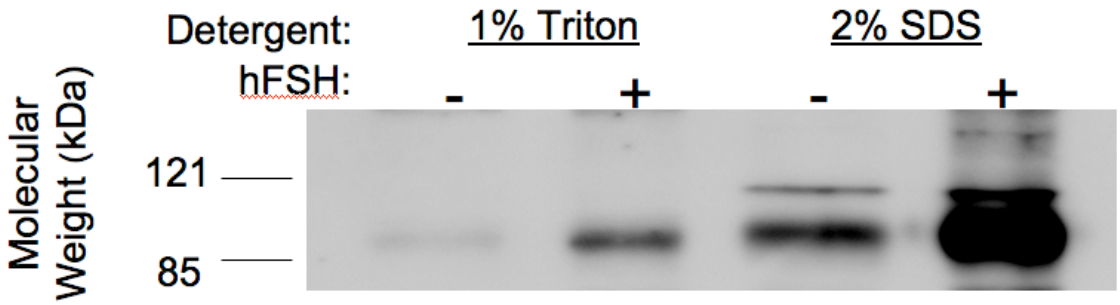

Figure 8: Cellular membrane fractions subjected to SDS-PAGE and western blot analysis using a monoclonal anti-FSHR antibody

In order to confirm the validity of the cellular membrane extraction process, Western blot analysis was performed for proteins that are known to reside in lipid rafts and those that are known to reside in the bulk membrane. Figure 9 represents western blot analysis probed with a monoclonal anti-Flotillin-2 antibody. Flotillin-2 is a membrane protein known to reside in lipid rafts in HEK 293 cells.[19] Flotillin-2 has a molecular weight of 42 kDa, which suggests that the bands observed in Figure 9 do represent the protein. The strongest band is present in the 2% SDS fractions. The bands in the 1% Triton fractions are faint.

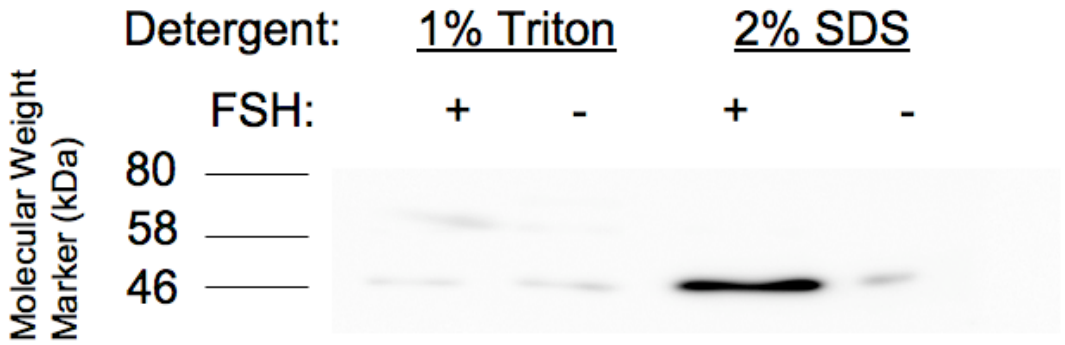

Figure 9: Cellular membrane fractions subjected to SDS-PAGE and western blot analysis using a monoclonal anti-flotillin-2 antibody

Western blot analysis was also conducted with a monoclonal anti-cadherin antibody. Cadherin is a membrane protein involved in cell adhesion that resides in lipid rafts in many cell types. Cadherin was detected at the appropriate molecular weight of 124 kDa (Figure 10).[20] These results show that cadherin was primarily found in the 2% SDS fractions. Less distinct bands are also present in the 1% Triton fractions.

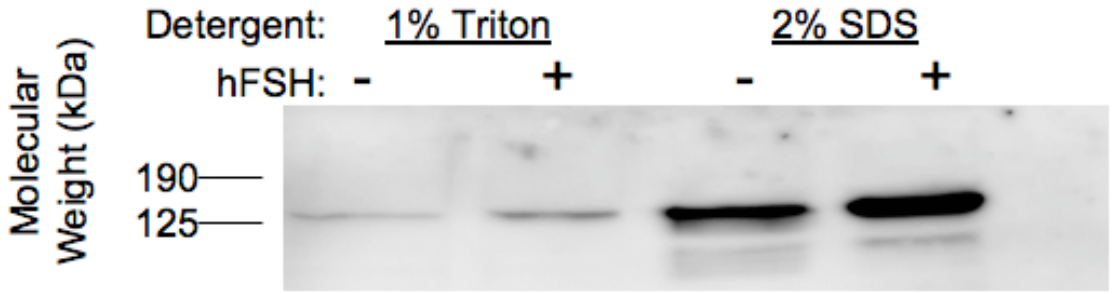

Figure 10: Cellular membrane fractions subjected to SDS-PAGE and western blot analysis using a monoclonal anti-cadherin antibody

Additionally, a monoclonal anti-gamma-tubulin antibody was used for western blot analysis of gamma-tubulin. Gamma-Tubulin is a cytoplasmic protein and component of centrosomes. It would therefore not be expected in lipid rafts.[21,22] Gamma-tubulin was detected at the appropriate molecular weight of 48 kDa (Figure 11). Discrete bands are present in the 1% triton fractions.

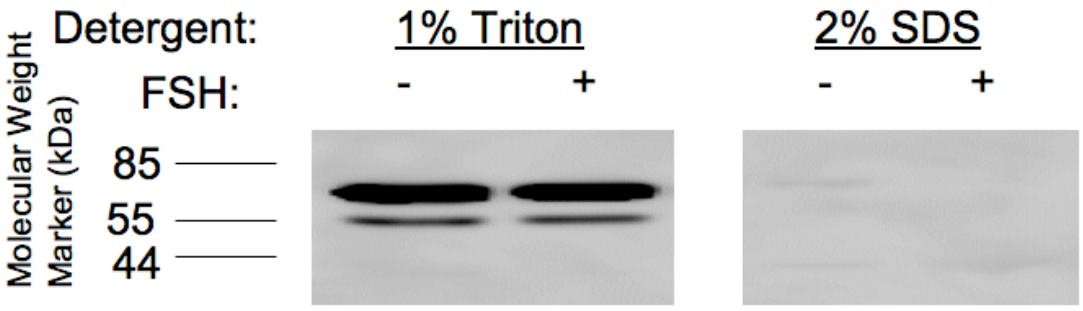

Figure 11: Cellular membrane fractions subjected to SDS-PAGE and western blot analysis using a monoclonal anti-gamma-tubulin antibody

MβCD treatment disrupted FSHR residency in 2% SDS fractions

Prior to cellular membrane extraction, HEK 293 hFSH were treated with MβCD (in addition to FSH). SDS-PAGE and western blot analysis were performed using a monoclonal anti-FSHR antibody. The 2% SDS fractions can be seen in Figure 12. There are no distinct bands in the 2% SDS fractions treated with FSH and MβCD. There are distinct bands in the remaining 2%SDS fractions which include no treatment with FSH and MβCD, just treatment with FSH, and just treatment with MβCD.

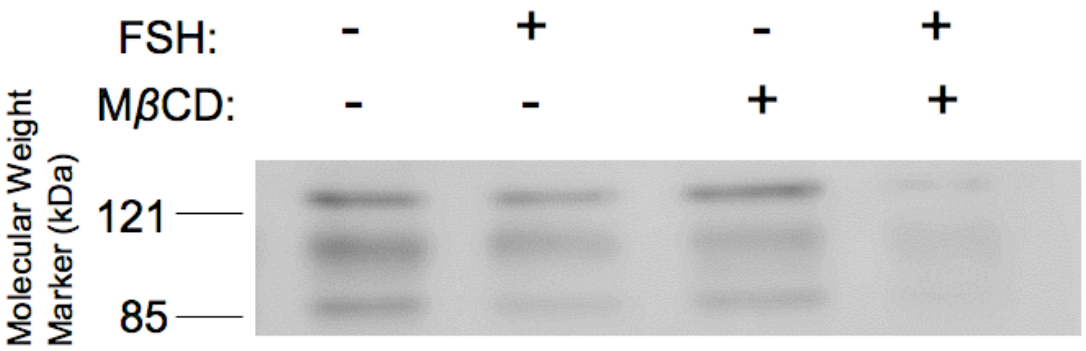

Figure 12: 2% SDS cellular membrane fractions treated with FSH and MβCD to disrupt lipid rafts. Fractions subjected to SDS-PAGE and western blot analysis using a monoclonal anti-FSHR antibody as described above.

DISCUSSION

Lipid rafts are densely packed microdomains that display a resistance to nonionic detergents due to the increased presence of cholesterol and sphingolipids with saturated acyl chains. Due to the resistance to nonionic detergents that is inherent to lipid rafts by definition, it can be concluded that the 2% SDS or detergent resistant fractions represent lipid rafts. It can also be concluded that the 1% triton or detergent susceptible fractions represent bulk membrane.

FSHR resides in both lipid rafts and bulk membrane

My results indicate that there are two populations of FSHR. The receptor is present at increased concentrations in the lipid rafts; however, FSHR is also present in the bulk membrane at decreased concentrations. Furthermore, treatment with FSH increases the residency of FSHR in lipid rafts. The methods for this investigation are supported by western blot analysis of membrane fractions probed with monoclonal antibodies to flotillin-2, cadherin, and gamma tubulin. Flotillin-2 is a marker protein for lipid rafts in HEK-293 cells, and these methods produced results that were consistent with this. Although a more pronounced band was expected in the lipid rafts fractions not treated with hormone, this inconsistency may be due to a transfer error during western blot analysis. Nonetheless, these results are strong indications that the method utilized to isolated lipid rafts was effective. Cadherin is also a protein that has been shown to reside in lipid rafts, and the results support this. Gamma tubulin is not a protein found in lipid rats, and the results show that gamma tubulin resided primarily in the bulk membrane.

Disruption of lipid rafts with MβCD alters FSHR residency in lipid rafts

When HEK-293 hFSHR cells were treated with M*β*CD, the normal pattern of FSHR residency in lipid rafts was altered. FSHR was no longer present at increased concentrations in lipid rafts. This finding suggests that FSHR resides in lipid rafts because if FSHR did not reside in lipid rafts, the distribution would not change. However, it would be expected that without M*β*CD treatment, most FSHR would then reside in lipid raft fractions upon FSH stimulation. However, the results are not consistent with this expectation.

The use of detergents to extract membrane fractions, specifically lipid rafts and caveolae, has been criticized. Eddin suggested that use of detergents for membrane fractionation can create membrane blebbing, fusion, and reorganization of lipids from their natural state. He also explored evidence that membrane solubilization with detergents could be temperature dependent.[23] All of these can cause inaccurate or inconsistent results. Due to these criticisms, inconsistencies of FSHR residency in lipid rafts in the experiments with MβCD, and the fact that my western blot analysis of membrane fractions probed with monoclonal antibodies to FSHR were not always as clear as those presented here, suggests that additional methods of membrane fractionation should be explored. Other methods of lipid raft isolation employ sodium carbonate to lyse cells and the use of a discontinuous sucrose gradient and ultracentrifugation to separate lipid rafts by density (sucrose density fractionation). [24, 25] Lipid rafts are found in the less dense, buoyant fractions in the sucrose gradient, which is most likely due to the high lipid to protein ratio.[26] Sucrose density fractionation, however, requires ultracentrifugation for 16 to 20 hours, and when used by Song et al, was found to have significant contamination of lipid raft fractions with other non lipid raft proteins such as transferrin receptor.[24] Another detergent free method conducted by Smart et al used an isotonic buffer with EDTA to lyse cells and a Percoll gradient to separate membrane fractions. Smart et al did not have as much contamination, but did experience poor yield.[24,26] Macdonald and Pike developed a fractionation method in which the buffer used to lyse the cells did not contain EDTA but instead contained the divalent cations magnesium and calcium. An Optiprep gradient and ultracentrifugation was then used to separate raft fractions from non-raft fractions. This last method appears to be fairly consistent, requires less time, and

does not show significant contamination. Macdonald and Pike suggested that the success of their detergent free method was due to the removal of EDTA. The divalent cations may stabilize the rafts such that they are more readily isolated. Detection of lipid raft proteins was done through western blot analysis in these varying detergent free methods.[24] None of these methods, however, were performed in HEK-293 cells. Comparison of the results of these detergent free methods of membrane fractionation and lipid raft isolation to the detergent fractionation employed in this study would validate the results obtained here.

Other areas to explore to further investigate FSHR residency in lipid rafts includes the mutagenesis of the putative caveolin interaction motif present in FSHR that may cause it to be located to the specific subset of lipid rafts known as caveolae. Furthermore, fluorescent microscopy techniques could be employed to observe if FSHR co-localizes with the caveolae marker protein caveolin with fluorescently tagged antibodies.

Understanding the residency of FSHR in membrane microdomains can help further knowledge in FSHR and GPCR signaling. This has important implications for human health. Specifically, FSHR and its signaling components and properties could be a pharmaceutical target for infertility treatment and contraception development, as previously mentioned. Furthermore, GPCRs are heavily involved in currently available pharmaceutical drugs, and increasing our understanding of how they work is beneficial.[27]

WORKS CITED

1. Holt, Richard, and Neil Hanley. *Essential Endocrinology and Diabetes (Essential)*. Grand Rapids: Blackwell Limited, 2006. Print.

2. Kumar, T. Rajendra, Yan Wang, Naifang Lu, and Martin M. Matzuk. "Follicle Stimulating Hormone Is Required for Ovarian Follicle Maturation but Not Male Fertility." *Nature Genetics* 15.2 (1997): 201-04.

3. Hunzicker-Dunn, M., and E. Maizels. "FSH Signaling Pathways in Immature Granulosa Cells That Regulate Target Gene Expression: Branching out from Protein Kinase A." *Cellular Signalling* 18.9 (2006): 1351-359.

4. "Ovulation Calendar, Ovulation Predictor,Endometrial Biopsy." *Top IVF Clinic India,IVF Infertility Clinic,IVF Fertility Center,In Vitro Fertilization*. Web. 06 June 2011. <http://www.drmalpani.com/book/chapter13a.html>.

5. Simoni, M. "The Follicle-Stimulating Hormone Receptor: Biochemistry, Molecular Biology, Physiology, and Pathophysiology." *Endocrine Reviews* 18.6 (1997): 739-73.

6. Chini B, and M. Parenti. "G-Protein coupled receptors in lipid rafts and caveolae: how, when and why do they go there?" *Journal of Molecular Endocrinology* 32 (2004) 325-338.

7. Oktay, K. "Ontogeny of Follicle-Stimulating Hormone Receptor Gene Expression in Isolated Human Ovarian Follicles." *Journal of Clinical Endocrinology & Metabolism* 82.11 (1997): 3748-751.

8. Ulloa-Aguirre, Alfredo, Teresa Zariñán, Ana Ma. Pasapera, Patricia Casas-González, and James A. Dias. "Multiple Facets of Follicle-stimulating Hormone Receptor Function." *Endocrine* 32.3 (2007): 251-63.

9. Alberts, Bruce. *Essential Cell Biology*. New York, N.Y.: Garland Science, 2010

10. Wayne, C. M., H.-Y. Fan, X. Cheng, and J. S. Richards. "Follicle-Stimulating Hormone Induces Multiple Signaling Cascades: Evidence That Activation of Rous Sarcoma Oncogene, RAS, and the Epidermal Growth Factor Receptor Are Critical for Granulosa Cell Differentiation." *Molecular Endocrinology* 21.8 (2007): 1940-957.

11. Ostrom, Rennolds S., and Paul A. Insel. "The Evolving Role of Lipid Rafts and Caveolae in G Protein-coupled Receptor Signaling: Implications for Molecular Pharmacology." *British Journal of Pharmacology* 143.2 (2004): 235-45.

12. Smith, S. M. L. "Luteinizing Hormone Receptors Translocate to Plasma Membrane Microdomains after Binding of Human Chorionic Gonadotropin." *Endocrinology* 147.4 (2005): 1789-795.

13. Patel, H. H., F. Murray, and P. A. Insel. *G-Protein-Coupled Receptor-Signaling Components in Membrane Raft and Caveolae Microdomains*. Handbook of Experimental Pharmolology 2008;(186):167-84.

14. Nelson and Cox, *Lehninger Principles of Biochemistry*, WH Freeman and Co, 4th edition, 2005.

15. Cohen et al. Unpublished data

16. Ostrom, Rennolds S., Steven R. Post, and Paul A. Insel.
"Stoichiometry and compartmentation in G protien coupled receptor signaling: implications for therapeutic interventions Involving G_s." *Perspectives in Pharmacology* 294.2 (2000): 407-412.

17. Hoekstra, Dick et al. "Membrane Dynamics and Cell Polarity the Role of Sphingolipids." *Journal of Lipid Research* 44 (2003): 869-77.

18. Cohen, B. D et al. "Regulation of Follitropin Receptor Cell Surface Residency by the Ubiquitin-Proteasome Pathway." *Endocrinology* 144.10 (2003): 4393-402.

19. Peiying Yu et al. "D1 Dopamine Receptor Signaling Involves Caveolin-2 in HEK-293 Cells." *Kidney International* 66.6 (2004): 2167-180. Print.

20. Seveau, Stephanie et al. "Role of Lipid Rafts in E-cadherin- and HGF-R/Met-mediated Entry of Listeria Monocytogenes into Host Cells." *The Journal of Cell Biology* 166.5 (2004): 743-53.

21. Wu Y et al. "DIXDC1 Co-localizes and Interacts with Gamma-tubulin in HEK293 Cells." *Cell Biology International* 33.6 (2009): 697-701

22. Starita, Lea et al. "BRCA1-Dependent Ubiquitination of Gamma-Tubulin Regulates Centrosome Number." *Molecular and Cellular Biology* 24.19 (2004): 8457-8466.

23. Edidin, Michael. "THE STATE OF LIPID RAFTS: From Model Membranes to Cells." *Annual Review of Biophysics and Biomolecular Structure* 32.1 (2003): 257-83.

24. Macdonald, Jennifer L., and Linda J. Pike. "A Simplified Method for the Preparation of Detergent Free Lipid Rafts." *Journal of Lipid Research* 46 (2005): 1061-06

25. Head, B. P. "Microtubules and Actin Microfilaments Regulate Lipid Raft/Caveolae Localization of Adenylyl Cyclase Signaling Components." *Journal of Biological Chemistry* 281.36 (2006): 26391-6399

26. Smart, E. J. "A Detergent-Free Method for Purifying Caveolae Membrane from Tissue Culture Cells." *Proceedings of the National Academy of Sciences* 92.22 (1995): 10104-0108.

27. Ja, William W., and Richard W. Roberts. "G-protein-directed Ligand Discovery with Peptide Combinatorial Libraries." *Trends in Biochemical Sciences* 30.6 (2005): 318-24.