Does Flotillin play a role in lipid raft organization of the GnRH receptor and its ability to transduce an intracellular signal?

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Introduction

Gonadotropin-Releasing Hormone (GnRH) and its subsequent signaling through the GnRH Receptor (GnRH-R) is critical for gonadal development and control of reproduction function. The GnRH-R is a member of the G-protein coupled receptor (GPCR) superfamily and is localized to specialized low-density areas on the cell membrane termed lipid rafts. These raft domains are implicated in GPCR coupled signaling by spatially organizing receptors and their associated signaling proteins to specific domains in the plasma membranes of cells. These raft domains appear to play an important role in the organization of GnRH-R and the signaling of GnRH to MAPkinase. Flotillin-1 is a protein thought to be intricately involved in the organization of rafts and the trafficking of proteins to raft domains. To examine the potential role of flotillin in GnRH signaling, gonadotrope derived αT3-1 cells were transfected with a specific siRNA for Flotillin-1 with the long-term goal of assessing the impact of Flotillin-1 deficiency on GnRHR trafficking to lipid rafts and signaling to intracellular targets including extracellular signal regulated kinase (ERK).

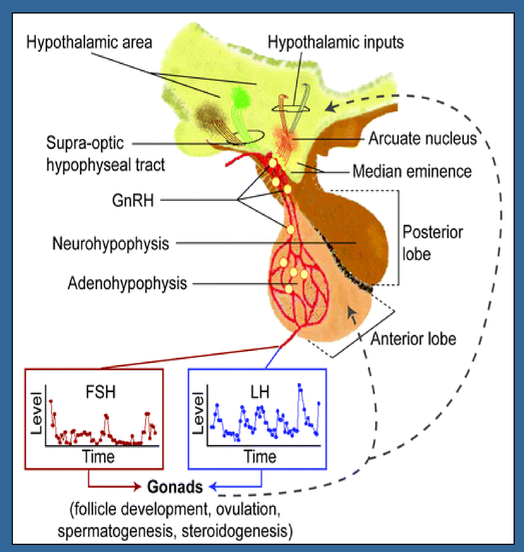

Fig 1. GnRH is synthesized by GnRH neurons in the hypothalamus and travels through the portal vasculature to the anterior pituitary gland, where it binds the GnRH receptor to stimulate the release of Luteinizing and Follicle Stimulating Hormones

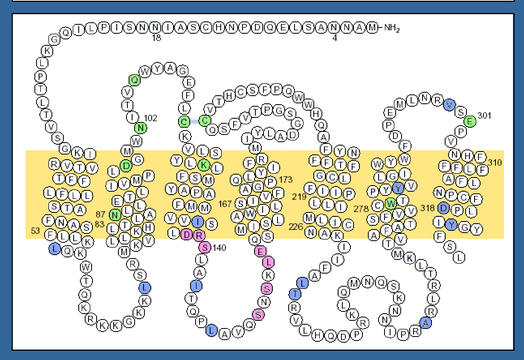

Fig 2. The absence of an intracellular carboxyl-terminus makes the GnRH-R an unusual member of the GPCR superfamily.

Objectives

- **To use si-*GLO* Red to determine the transfection efficiency of Flotillin-1 siRNA**
- **To use siRNA technology to knockdown Flotillin-1 expression**
- **To determine the effect of Flotillin-1 knockdown on GnRH-R signaling**

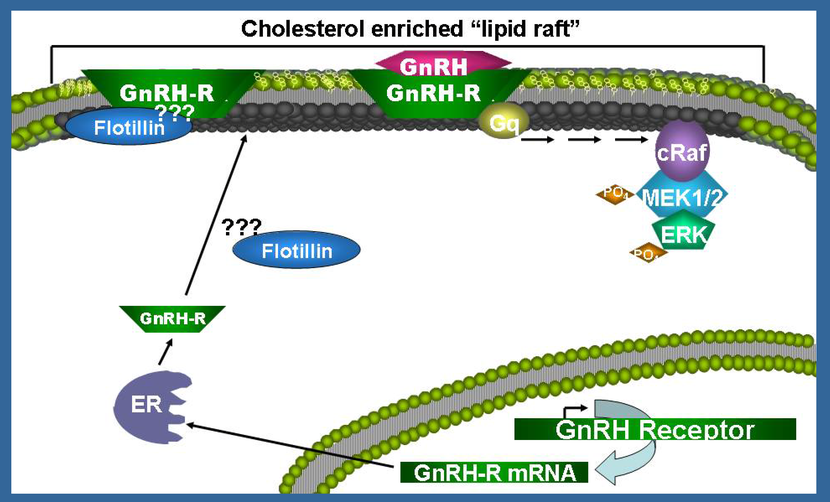

Fig 3. Experimental question: Is flotillin-1 involved in directing GnRH-R localization to cholesterol enriched membrane rafts?

Materials and Methods

- αT3-1 cells were grown to confluence and transfected with si*GLO*, an siRNA indicator, along with specific Flotillin-1 siRNA at a concentration of 40 nM and examined using confocal microscopy for red fluorescence to determine transfection efficiency
- Electrophoretic separation was performed on transfected cell lysates followed by Western blot analysis. Membranes were probed for Flotillin-1 or phosphorylated (activated) extracellular signal regulated kinase (ERK), an established protein in the GnRH signaling pathway

Results

- **Expression of siGLO indicator is evident in transiently transfected αT3-1 cells.**

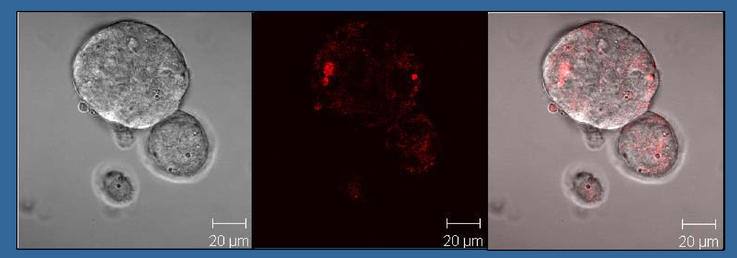

Fig 4. Confocal microscopy images of αT3-1 cells transfected with si*GLO* and Flotillin-1siRNA.

- **Attenuated expression of flotillin is evident following transfection of αT3-1 cells with Flotillin-1 siRNA.**

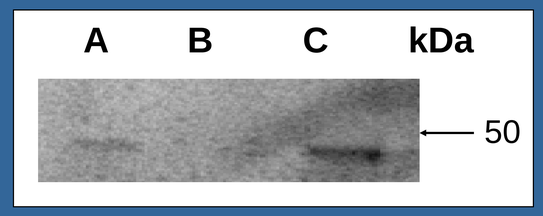

Fig 5. Western blot probed with anti-Flotillin-1, which has an expected size of 47 kDa. Sample A is αT3-1 whole cell lysate, sample B is Flotillin-1 knockdown + si*GLO,* sample C is si*GLO* only.

Future Studies

- To define the optimal time course of siRNA expression and optimize siRNA concentrations
- To determine the effect of Flotillin-1 knockdown on GnRH meditated ERK phosphorylation
- To determine if Flotillin-1 knockdown disrupts GnRH-R trafficking to lipid rafts