Abstract

My project investigates the function of the FSHR-1 protein in regulating synaptic transmission at the neuromuscular junction (NMJ) in *C. elegans* roundworms by examining FSHR-1 as a possible neuropeptide receptor and its involvement in G-protein signaling pathways. This will provide new information regarding the potential neuronal functions of FSHR-1 and help our understanding of neuropeptide functions. I will perform behavioral assays measuring the amount of signaling for muscle contraction in worms having mutations (*egl-21, egl-3, unc-31*) affecting neuropeptide signaling, as well as other worms having mutations (*gsa-1, acy-1*) affecting downstream signaling of FSHR-1. I hypothesize that FSHR-1 is a neuropeptide receptor modulating synaptic transmission at the NMJ presynaptically. Neuronal proteins, including FSHR-1, are important because they are highly conserved between humans and *C. elegans*; thus my results will contribute new insights into the role of neuropeptides, as they are good drug targets for human diseases such as Parkinson's and Alzheimer's.

Significance of Project

Our body is controlled by the nervous system. Information is received by our senses, processed in the brain and sent back out to perform a response. The circuitry of this communication network is composed of neurons that connect at junctions called synapses. It is at theses synapses that chemical signaling occurs in which small molecules travel across the synaptic cleft to relay information from the presynaptic ("sending") neuron to the postsynaptic ("receiving") neuron. Specifically, neurotransmitters (the chemical signals) are released from the presynaptic neuron upon arrival of an electrical signal in the neuron. The neurotransmitter then causes an excitatory or inhibitory response in the postsynaptic neuron by binding to receptors on that cell. This binding can cause either further transmission of the signal (excitatory) or can prevent the signal from spreading (inhibitory) into the postsynaptic cell.

A regulation of excitatory and inhibitory signaling, called synaptic transmission, is critical as a balance between the two allows for normal neuronal function. There are hundreds of synaptic proteins that regulate synapse function presynaptically and postsynaptically, but how they directly regulate synaptic transmission is not fully understood. Misregulation that occurs between excitatory and inhibitory signaling is among the initial effects of neurodegenerative disorders such as Parkinson's disease or Alzheimer's.[1] Thus, understanding the factors involved in controlling signaling at neuronal synapses is important for being able to better treat these disorders.

Besides neurotransmitters other regulatory molecules, neuropeptides, are released from presynaptic neurons. Neuropeptides are short sequences of amino acids that can function directly or indirectly to modulate synaptic transmission. There are over 250 neuropeptides, identified to date, that work at synaptic clefts. Neuropeptides can be categorized into two different families: insulin-like peptides, and the FMRFamide-related peptides (FLPs).[2] Neuropeptides work to auto-regulate the presynaptic neuron by modulating the amount of neurotransmitters released into the synaptic cleft. Neuropeptides also regulate the postsynaptic neuron by modulating the number and type of neurotransmitter receptors present on that neuronal cell. Neuropeptide receptors are most often G-protein coupled receptors (GPCRs). There are four main types of G-protein pathways that may be activated by these GPCRS: Gαq, Gα12, Gαo, and Gαs.[3] There is still a lot unknown about the specificity of neuropeptides and their receptors and the cellular pathways and effects they activate. In addition, continued research is needed in order to better understand the specific synapses and processes in which neuropeptides act to modulate synaptic transmission.

Given the complexity of the human nervous system, to study these questions related to the control of synaptic transmission, I will be using *C. elegans* roundworms in Dr. Kowalski's lab. *C. elegans* are excellent animals in which to perform these studies because there are many similarities between the human and *C. elegans* nervous systems, particularly in terms of their protein composition and function and the basic circuitry of the nervous system. However, the *C. elegans* nervous system is less complex, and all of the synaptic connections are known. These worms also have a set of simple behaviors, allowing for easier manipulation and measurement of synaptic transmission through behavioral assays in the lab. In addition, *C. elegans* have short life cycles that allow for easy breeding and manipulation and are excellent for the genetic studies I am proposing.

Our lab uses the *C. elegans* neuromuscular junction (NMJ) a model synapse for studying synaptic transmission (Figure 1). The NMJ is the synapse between a presynaptic neuron and a postsynaptic muscle, which ultimately controls muscle contraction. Contraction at the *C. elegans* NMJ is controlled by a balance of excitatory (acetylcholine) and inhibitory (GABA) signaling. The amount of signaling for muscle contraction can be measured by a behavioral assay using a chemical called aldicarb. Aldicarb is a reversible acetylcholinesterase inhibitor. Acetylcholinesterase is found in the synaptic cleft of *C. elegans* and works to break down the neurotransmitter acetylcholine (Ach), thus acetylcholinesterase works to shut off muscle contraction at the NMJ. By subjecting the worms to aldicarb, it causes paralysis due to muscle hyper-contraction as a result of accumulation of acetylcholine in the synaptic cleft.[4] Worms that have mutations that cause an increase in synaptic transmission (Ach) will paralyze faster than wild type worms. The opposite is true, as well: worms that have mutations that cause a decrease in synaptic transmission (Ach) will paralyze more slowly.[4] The aldicarb assay has been used extensively by Dr. Kowalski's lab, as well as others, to identify genes involved in synaptic transmission at the NMJ.

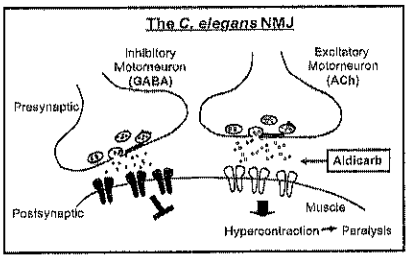

Figure 1. Acetylcholine and GABA signaling work to control contraction and relaxation at the *C. elegans* NMJ. Aldicarb causes acetylcholine to build up leading to paralysis.

My proposed research focuses around role of one gene that is required for normal NMJ signaling, FSHR-1, and the mechanisms by which it affects synaptic transmission at the *C. elegans* NMJ. FSHR-1 is a G-protein coupled receptor that is required for innate immune response and germline differentiation in *C. elegans*.[5] FSHR-1 loss of function mutants exhibits increased resistance to aldicarb compared to wild type, and current students working in Dr. Kowalski's lab have identified FSHR-1 as a possible substrate of the Anaphase Promoting Complex (APC) enzyme, another regulator of NMJ signaling (unpublished data). FSHR-1 is the only *C. elegans* protein similar to the follicle stimulating, thyroid stimulating and luteinizing hormone receptor in mammals; however, recent research has suggested that FSHR-1 is a neuropeptide receptor.[6] In addition, another study provides evidence that FSHR-1 possibly functions presynaptically in motorneurons since its expression is not seen in muscle, but rather occurs at high levels in the intestines and neurons of *C. elegans* and since animals lacking FSHR-1 have decreased synaptic vesicle release in acetylcholine neurons.[7] Finally, a recent study demonstrated that the Gαs pathway may be activated downstream of FSHR-1 in germline differentiation; however, nothing is known about pathways it activates in neurons. My proposed project will investigate the role of FSHR-1 in neuropeptide signaling as well as the downstream signaling pathways it activates to control NMJ signaling.

Statement of Research Objectives

The goal of this project is to determine how FSHR-1 regulates synaptic transmission at the *C. elegans* NMJ. Specifically, I will investigate (1) the potential involvement of *fshr-1* in neuropeptide signaling, which has been shown to impact NMJ signaling, and (2) the downstream pathways by which FSHR-1 may control muscle contraction at the NMJ. Based on data from previous studies, including the high expression of *fshr-1* in neurons, its

effects on synaptic vesicle release in acetylcholine neurons, and its apparent ability to signal through the Gαs pathway to regulate germline differentiation, I hypothesize that *fshr-1* is a neuropeptide receptor that acts in presynaptic motorneurons by signaling through the Gαs pathway to promote muscle contraction at the NMJ.

Methods

Question 1: Is FSHR-1 *involved in neuropeptide signaling to control NMJ transmission?*

Previous studies have suggested that FSHR-1 may be acting as a neuropeptide receptor to regulate NMJ signaling.[6] Other studies have shown that worms lacking the function of essential neuropeptide processing and release genes are aldicarb resistant, similar to the phenotype seen with FSHR-1 loss of function worms.[6] If FSHR-1 is regulating muscle contraction at the NMJ in response to neuropeptide signaling occurring in the same pathway, then animals lacking both neuropeptide signaling and FSHR-1 should show a non-additive phenotype in the aldicarb assay (Figure 2). A non-additive phenotype result would be one in which the aldicarb resistance for a double mutant is not worse or better than either single mutant phenotype. If instead, FSHR-1 and neuropeptides control muscle contraction independently, then animals lacking both genes should have a stronger aldicarb resistance than animals lacking only one or the other, showing an additive relationship.

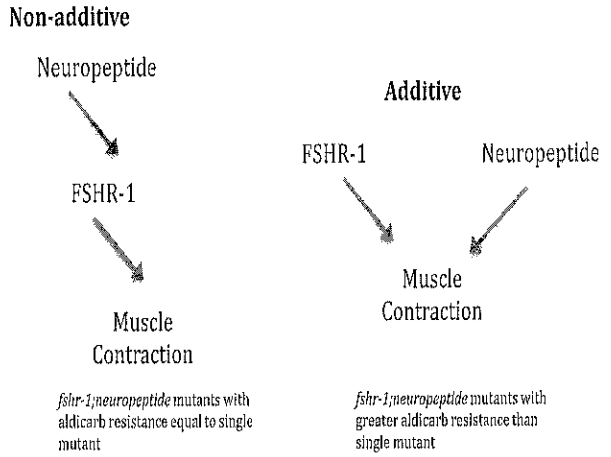

Figure 2. Neuropeptides and FSHR-1 may be working in the in the same pathway, non-additive, or different pathway, additive, to affect muscle contraction.

Data have shown that UNC-1 (CAPS) is required for the release of dense-core vesicles (neuropeptides). Specifically, worm with loss of function mutations in the *unc-31* CAPS gene have been shown to be resistant to aldicarb-induced paralysis. In addition, the *egl-21* and *egl-3* genes both encode proteins that are expressed in the nervous system and are known as neuropeptide processing enzymes. Some research demonstrates that mutant worms that are deficient in *egl-21* or *egl-3* have diminished acetylcholine release at the NMJ because of reduced movement of neuropeptide-containing vesicles in acetylcholine neurons.[8] To test whether FHSR-1 and neuropeptides act in the same pathway to control muscle contraction, I will generate double mutants between *fshr-1* and each of these neuropeptide single mutants (*fshr-1;egl-21* or *fshr-1;egl-3,* and *fshr-1;unc-31)* and test these animals in the aldicarb assay relative to the wild type and to each single mutant. Synaptic transmission will be quantified using rate of paralysis in the aldicarb assay. In each experiment, aldicarb assays will be done in triplicate with 20 worms per plate.

If I observe non-additive affects with *egl-21*, *egl-3*, or *unc-31* I will conclude that *fshr-1* is working in the same pathway, thus *fshr-1* is likely involved in neuropeptide signaling. If additive, then *fshr-1* is likely not action in neuropeptide pathway. I will then focus on downstream signaling (question #2). We have all of these mutant strains currently in the lab, and I will be building double mutants in lab this spring in order to be prepared to begin aldicarb

experiments this summer. Further, if I find that FSHR-1 controls NMJ transmission through the same genetic pathway that neuropeptides work, I will then be able to test individual neuropeptides to look for a ligand, specifically *ins-22*, *ins-21*, *flp-1*, and *nlp-12*.[6] These neuropeptides were identified as being required for NMJ signaling in the same study that first identified FSHR-1 as an NMJ regulator.[6]

Question 2: What downstream signaling pathway does FSHR-1 activate to control NMJ signaling/muscle contraction?

It has been shown that *fshr-1* loss of function mutants have a resistance to aldicarb-induced paralysis, demonstrating that FSHR is necessary for normal synaptic transmission at the NMJ. [6] In a previous study investigating the role of FSHR-1 in germline differentiation, the Gαs pathway was shown to be involved downstream of FSHR-1.[5] Specifically, gain of function mutations in Gαs (*gsa-1*) or another downstream enzyme in the pathway *acy-1*, were able to rescue defects in germline different caused by loss of *fshr-1* function.[5] Moreover, loss of function of *gsa-1* and *acy-1* causes decreased synaptic transmission (aldicarb resistance) as does *fshr-1*.[6] Thus, it likely *fshr-1* acts through this G-protein pathway to regulate NMJ signaling.

I will test this by generating a series of double mutants between *fshr-1* and these loss- (lof) and gain- of function (gof) G-protein pathway genes: *fshr-1;gsa-1*(gof), *fshr-1;gsa-1*(lof), *fshr-1;acy-1* (gof), and *fshr-1;acy-1* (lof) which I will then test relative to wild type and single mutants and quantify the results based on an aldicarb assay. If *fshr-1* and *gsa-1* or *acy-1* are functioning in the same pathway, then non-additive aldicarb phenotypes should be seen in double mutants relative to the single mutants (Figure 3). However, additive aldicarb phenotypes would occur if *fshr-1* and *gsa-1* or *acy-1* work in independent pathways. I will test the double and single mutants against wild type *C. elegans*. In each experiment, aldicarb assays will be done in triplicate with 20 worms per plate.

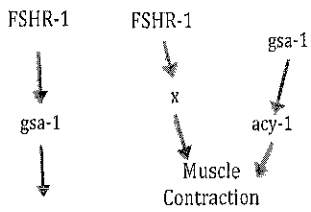

Figure 3. FSHR-1 may be working in the same G-protein pathway (non-additive) as gsa-1 and acy-1 to control muscle contraction. FSHR-1 could be working in a different pathway as gsa-1 and acy-1 (additive).

For the experiments with the gain of function pathway mutants, *fshr-1;gsa-1(or acy-1)* gof double mutants should show increased aldicarb sensitivity that would match that of the gof *gsa-1* mutants if these genes function in the same pathway to control muscle contraction. This is expected since the gof mutants would be able to restore the function of the pathway in spite of the lack of *fshr-1*. For the experiments with the loss of function G-protein mutants, the *fshr-1;gsa-1(or acy-1)* lof mutants should show aldicarb resistance that is no greater than either single mutant. However, if *fshr-1* and *gsa-1* genes are functioning in different pathways, then *fshr-1;gsa-1* (gof) animals should show intermediate phenotypes that are similar to wild type, whereas the *fshr-1;gsa-1* lof mutants should show increased resistance to aldicarb versus single mutants(Figure 3).

If the Gαs pathway is found to be acting independently of FSHR-1 to control muscle contraction, then I will test if FHSR-1 acts with other G-protein pathways by again performing double mutant analysis between *fshr-1* and the following G-protein mutants: *goa-1, gpa-12, and egl-30* (Gαq pathway). We have access to all of these strains. Thus, I should be able to successfully determine downstream pathways of FSHR-1 important at the NMJ.

Conclusion

Successful completion of the above experiment will provide new information about the function of FSHR-1 and its signaling pathways in neurons. It is important to understand if FSHR-1 is involved in neuropeptide signaling because little is known about neuropeptides and their role in synaptic transmission. In addition, G-protein coupled receptors, like FSHR-1, are abundant in *C. elegans* and humans and are important drug targets, therefore a vital area of research that could open doors to cures to diseases such as Parkinson's or Alzheimer's.

References

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Progression of Project

Week 1-4: Building and confirming *fshr-1* neuropeptide and *fshr-1* signaling pathway mutants.

Week 5-8: Testing double mutants against controls in aldicarb assay.

Week 9: Prepare and share BSI presentation

Feasibility

During my project I will use a PCR machine and a camera to take gel pictures. I will have access to departmental PCR machines and the departmental AlphaImager gel camera located in the basement of Gallahue. I will also be using a fluorescent dissecting microscope, an injection microscope, and DNA gel electrophoresis equipment. This equipment as well as *C. elegans* worm strains and incubators, and all other lab equipment I need will be located in Dr. Kowalski's lab. During the spring 2012, I will be volunteering several hours a week in Dr. Kowalski's lab so I will be familiar with the lab techniques. Any unfamiliar techniques or methods will be taught to me by fellow lab members or by Dr. Kowalski. I will also begin preliminary experiments on my proposed project.