Undergraduate Research Symposium May 16, 2014 Mary Gates Hall

Online Proceedings

POSTER SESSION 1

Commons East, Easel 82

11:00 AM to 1:00 PM

The Role of the PDZ-binding Motif in Somatostatin Receptors

Marianne Elizabeth (Marianne) Estrada, Junior, Biochemistry
Mentor: Chris Hague, Pharmacology, University of Washington School of Medicine

The development of drugs is essential for human health. Approximately 40-60% of all currently prescribed drugs target a specific type of membrane protein called a G-protein coupled receptor (GPCR). In the human body, the binding of a neurotransmitter or hormones to a GPCR transmits signals across the membrane to bring about an intracellular response, which ultimately permits cells to communicate with each other and regulate body physiology. The Hague lab studies the molecular mechanism of drugs targeting GPCRs. Currently, we are investigating an important structural portion of certain GPCRs called the PDZ binding motif, which is a site for protein-protein interactions. My role in the lab focuses on the somatostatin (SST) receptor subfamily, which regulates the endocrine system and neurotransmission by inhibiting the release of secondary hormones produced in the gastrointestinal tract and pituitary gland. A drug that targets SST receptors is octreotide. This drug inhibits growth hormone and insulin release, and is FDA approved for the treatment of growth-hormone producing tumors and GI tract disorders. Interestingly, all five SST GPCRs contain this PDZ-binding motif, and my research examined the importance of this motif using a variety of biochemistry techniques that quantified the expression, localization, signaling, and protein-protein interaction network. I created SST receptor mutants lacking the PDZ-binding motif using polymerase chain reaction and then transfected the SST mutants into human cells. These novel clones were subjected to plasma membrane assays, confocal microscopy, and mass spectrometry analysis. I found that removing the PDZ-binding motif had minor impact on overall receptor function, but more studies are necessary to determine the role of the PDZ-binding motif for SST receptor function. Our ultimate goal is to identify novel PDZ-interacting proteins for GPCRs, and then disrupt or enhance this interaction with novel small molecules to treat disease.

POSTER SESSION 1

Commons East, Easel 83

11:00 AM to 1:00 PM

Characterizing the PDZ-Binding Motif in Serotonin Receptors

Dorathy-Ann (Dory) Harris, Senior, Neurobiology
EIP Scholar, Initiative for Maximizing Student Development Scholar, McNair Scholar
Mentor: Chris Hague, Pharmacology, University of Washington School of Medicine
Mentor: Kyung Soon Lee, pharmacology
Mentor: Jennifer Wacker Mhyre, Pharmacology

The development of new medications is critical for treating disease and extending life. Approximately 40-60% of drugs target a specific type of membrane protein termed G-Protein Coupled Receptors (GPCRs), which permit cells to communicate with each other (by binding neurotransmitters) and regulate body physiology. The Hague lab studies the molecular mechanism of drugs targeting these receptors. Specifically, they examine a small, yet important structural portion of the receptor - the PDZ binding motif - which acts a protein-protein interaction site. Interestingly, of the ˜800 different GPCRs in the body, 30 have this PDZ binding motif, including those activated by the neurotransmitter 5-hydroxytryptamine (5-HT), which is commonly known as serotonin. Serotonin regulates many key CNS processes including mood, sleep, and appetite. Current 5-HT medications that are on the market are used to treat depression, generalized anxiety disorder, and social phobia. Two common antidepressants that affect serotonin GPCRs are Selective Serotonin Reuptake Inhibitors, or SSRIs, and Monoamine Oxidase Inhibitors, or MAOIs. My goal was to examine the necessity of the PDZ binding motif for 5-HT serotonin receptor function in human cells. To do this, HEK293 cells expressing the wild-type 5HT receptor (WT) or mutated 5-HT receptor missing the PDZ binding motif (ΔPDZ) were subjected to diagnostic assays including: microscopy, protein biochemical analysis, proteomics, and cellular signaling. In summary, the data suggest the PDZ-binding motif is not essential for 5-HT serotonin receptor function in HEK293 cells, although further experiments are necessary to solidify this conclusion.

POSTER SESSION 2

Balcony, Easel 120

1:00 PM to 2:30 PM

Cognitive Effects of Silencing Dopamine Receptor-1 Expressing Cells in Dentate Nucleus of Cerebellum in Mice

Julia Licholai, Senior, Neurobiology
Mentor: Larry Zweifel, Psychiatry and Pharmacology
Mentor: Erik Carlson, Psychiatry and Behavioral Sciences

Cerebellar pathology has been associated with cognitive and affective deficits and cerebellar dysfunction may play a role in schizophrenia and autism spectrum disorder. Understanding how cerebellar abnormalities contribute to characteristic features of psychiatric disorders may pave the way to creating better therapies. One cerebellar output region, the lateral dentate nucleus (LDN), is associated with cognitive and affective roles in behavior. This region has reciprocal connections with the limbic system, where dopaminergic neurotransmission has been linked with psychiatric disorders. Cells expressing dopamine receptor-1 (D1R) can be found in the LDN, but their function is unknown. To determine whether D1R-expressing neurons in the LDN modulate cognitive, affective, and/or social behavior we used a pharmacogenetic approach in mice. Specifically, we conditionally expressed a yellow-florescent-protein–tagged HM4Di, the inhibitory DREADD Receptor (designer receptors exclusively activated by designer drugs) or green florescent protein (GFP) in D1R-expressing cells of the LDN by injecting mice expressing Cre recombinase, an enzyme used for precise gene expression manipulation, under control of the endogenous D1R gene (Drd1a$^{C}re/+$) with conditional viral vectors. Systemic injection of the DREADD-specific ligand, Clozapine-N-Oxide (CNO), reversibly inhibits HM4Di-expressing cells allowing us to test their function in behavior. In the experiments I performed, in the presence of CNO, Drd1a$^{C}re/+$; DNC-HM4 mice showed improved motor performance, decreased prepulse inhibition of acoustic startle reflex (a reflexive jolt triggered by sound), increased anxiety-like behavior in an elevated plus maze, reduced cognitive performance in a spatial navigation memory task, and impaired differentiation of novel and familiar mice compared to controls. No significant differences were seen between groups in an instrumental conditioning experiment, a measure of motivation for food reward. D1R-expressing neurons in the LDN influence specific cognitive, affective, and social behaviors. Alterations in dopaminoceptive neurons within specific cerebellar nuclei may play important roles in the etiology of symptoms of mental illness.

SESSION 2E

NEW TOOLS FOR EXPLORING PROTEIN FUNCTION, STRUCTURE AND PATHOLOGY

Session Moderator: Douglas Fowler, Genome Sciences

238 MGH

3:30 PM to 5:00 PM

* Note: Titles in order of presentation.

N-Terminal Cleavage of the Alpha 1-D Adrenergic Receptor

Timothy Steven (Tim) Kountz, Senior, Biochemistry
Mary Gates Scholar, UW Honors Program
Mentor: Chris Hague, Pharmacology, University of Washington School of Medicine

In the Hague Lab we study a class of proteins called G-protein coupled receptors (GPCR's). GPCR's are seven-pass transmembrane proteins that are involved in cell signaling. One GPCR we study is called alpha-1D adrenergic receptor. Adrenergic receptors bind the endogenous catecholamines adrenaline and noradrenaline. An important functional role of the alpha-1D GPCR in the body is to regulate blood pressure by affecting blood vessel diameter. Our lab recently discovered that the alpha-1D adrenergic receptor's relatively long N-terminal domain is cleaved in human cells. We are currently elucidating the specific cleavage site - which we suspect is a GL matrix metalloprotease domain - and the physiological purpose of the cleavage. Over the last few months we have narrowed down the region of cleavage to approximately 15 amino acids. We also have a working theory that the N-terminal cleavage of the protein allows for better cell surface localization. We are currently examining how the cleavage event effects the signaling characteristics of the protein.

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Developing Tools to Study Mechanisms of k-Opioid Receptor Activation of c-Jun-N-terminal Kinase

Talia Suner, Senior, Neurobiology, Biochemistry
NASA Space Grant Scholar, UW Honors Program
Mentor: Charles Chavkin, Pharmacology
Mentor: Selena Schattauer, Pharmacology

It has been shown that the chemical nor-binaltorphimine (norBNI) inactivates the k-opioid receptor (KOR) for days or weeks in vivo, and also results in downstream activation of c-Jun N-terminal kinase (JNK). If JNK is blocked in vivo by SP610025 or by knocking out the JNK1 gene, norBNI inactivates the KOR for just hours. We hypothesize that there is a JNK mediated inactivation of the KOR. To further investigate this, I will create constructs that will allow us to study the interactions of JNK and arrestin, two proteins that are involved in the transduction of KOR signaling. I aim to express a version of each JNK1 splice variant tagged with luciferase and arrestin3 tagged with Venus in human embryonic kidney (HEK293) cells that have been transfected with rat KOR. Venus and luciferase are two bioluminescent molecules that emit different wavelengths of light depending on their distance from one another. After treating these cells with norBNI, I can dtermine the level of their interaction by using a bioluminescence resonance energy transfer (BRET) assay. These experiments will provide useful data in determining the extent of the interactions between JNK1 and arrestin3. This data will help us understand the protein cascade that results in the deactivation of the KOR, and if certain drugs activate certain splice variants. Understanding this pathway has implications in creating analgesics with smaller potential for abuse and addiction, and creating pharmaceuticals that could help with stress related depression and addiction relapse.

POSTER SESSION 4

Commons East, Easel 51

4:00 PM to 6:00 PM

Genetic Variability in CRHR1 and its Association with Participant Response to Glucocorticoid Receptor-Mediated Signaling

Olivia Fox, Junior, Biology (Molecular, Cellular & Developmental), Pre-Nursing
Mentor: Patrick Murphy, Interdisciplinary Health Sciences, Seattle University

Pharmacogenetics is the field of biomedical science that studies how variation in a person's genetic composition affects his or her response to drugs. Glucocorticoids are a group of signaling molecules that include endogenous hormones such as cortisol, which the body produces naturally in response to various forms of stress, as well as drugs with strong anti-inflammatory properties that are used to treat diseases ranging from asthma to cancer. The goal of this study was to identify correlations between interpersonal genetic variability and alterations in glucocorticoid receptor (GR)-mediated signaling. Salivary cortisol concentrations were measured in healthy volunteers (n=66) in order to determine differences in circulating cortisol concentrations and cortisol response following simulated physiological stress. Statistical analysis indicated inter-participant cortisol (CORT) variability was not correlated to participant demographics, health history, or single nucleotide polymorphisms (SNPs) in genes encoding the GR (NR3C1) or the essential GR chaperone protein hsp90 (HSP90AA1). Two participants with highly similar demographic profiles and opposing CORT responses (a matched pair) were selected for exome sequencing—the sequencing of all coding regions in the genome. A filtering algorithm identified candidate SNPs in approximately 30 genes involved in mediating the cortisol response and GR signaling. Interestingly, corticotropin releasing hormone receptor 1 (CRHR1) showed a difference of over 20 SNPs between the matched exome pair. CRHR1 regions were then sequenced from the larger participant group. Participants with average cortisol concentrations that slightly decreased following CORT stimulation most commonly exhibited a unique SNP profile—they shared many of the same SNPs in CRHR1. We speculate participants with SNP profiles associated with altered cortisol response may share a corresponding sensitivity to corticosteroid-based drug therapies.

POSTER SESSION 4

Commons East, Easel 83

4:00 PM to 6:00 PM

Learning and Memory: Investigating the Role of Circadian Rhythm in Hippocampal Adult Neurogenesis

Sarah A. Larsen, Fifth Year, Neurobiology
UW Honors Program
Mentor: Daniel Storm, Pharmacology
Mentor: Sarah Wardlaw, Neurobiology & Behavior

Learning and memory are influenced both by adult neurogenesis and circadian rhythmicity; however, it is unclear if and how the biological clock influences neurogenesis. The BMAL1 protein is an essential component in the molecular clock. Transgenic Bmal1 -/- (knock out) mice, which lack the gene to produce this protein, are completely arrhythmic. Furthermore, the Bmal1 knockout mouse demonstrates poor learning and memory compared to its wild type littermate control which retains both Bmal1 alleles. As an animal that is both arrhythmic and exhibits deficits in learning and memory, the Bmal1 knockout mouse provides the opportunity to investigate the potential interaction of the molecular clock and neurogenesis. I hypothesized that the rate of adult neurogenesis in the hippocampus of the Bmal1 knockout mouse would be reduced as compared with wild type littermate controls. To quantify this, I used immunohistochemistry to fluorescently

mark proliferating cells with bromodeoxyuridine (BrdU) in the subgranular zone (SGZ) of the hippocampus. BrdU is a synthetic nucleotide, intraperitonealy (IP) injected prior to brain collection, and incorporated into the DNA of newly dividing cells. I found that the Bmal1 knockout hippocampus had significantly reduced rates of cell proliferation and survival compared with wild type controls. Thus, BMAL 1 is required for adult neurogenesis in the hippocampus. This finding now invites the consideration of whether BMAL 1 protein plays a direct cellular role in adult neurogenesis, or if it is the disruption to systemic circadian rhythmicity which compromises neurogenesis.

POSTER SESSION 4

Commons West, Easel 13

4:00 PM to 6:00 PM

Nalfurafine, a Drug used to Alieviate Itch in Hemodialysis Patients, Activates the Kappa Opioid Receptor to Inhibit Pruritic Effects of 5'-GNTI

Allisa Song, Senior, Biology (Physiology), Psychology
Mentor: Charles Chavkin, Pharmacology
Mentor: Selena Schattauer, Pharmacology

The aim of this study was to further the understanding of mechanisms that underlie the pruritic and antipruritic effects of kappa opioid receptor drugs. The kappa opioid receptor (KOR) has been studied for its potential therapeutic benefits. It has been previously shown that the kappa opioid receptor antagonist, 5'-guanidinonaltrindole (GNTI), causes compulsive, hind-leg scratching in mice, while the kappa opioid receptor agonist, nalfurafine, inhibits this pruritic effect. There are applications for this antipruritic effect in medicine, to counteract symptoms in kidney-failure patients. By learning more about the mechanisms of this anti-pruritic effect, better drugs can be developed in the future with increased specificity and decreased side effects. We pre-treated male C57/BL6 mice with either saline or nalfurafine (0.05mg/kg, s.c. flank), and challenged them 20 minutes later with 5'-GNTI (0.03mg/kg, s.c. behind the neck) or saline. The number of hind-leg scratches in the 30 minutes following GNTI injection were counted as the measure of itch. We compared the results between kappa opioid receptor (KOR) knock-out mice, mu opioid receptor (MOR) knock-out mice, and wild-type mice, to determine the role of KOR and MOR in GNTI-induced itch and nalfurafine inhibition of itch. We concluded that while the pruritic effects of 5'-GNTI are not mediated by KOR or MOR, the antipruritic effects of nalfurafine was KOR dependent.

POSTER SESSION 4

Commons East, Easel 62

4:00 PM to 6:00 PM

Molecular Characterization of the Inhibitory Function of TAF7 in TFIID-Mediated Transcription Regulation

Ariana Kamaliazad, Senior, Biology (Physiology)
Mentor: Edith Wang, Pharmacology

In eukaryotic organisms, regulation of gene transcription depends on the presence or absence of transcription factors, which are proteins that bind to promoter sequences on DNA. One common promoter element is the TATA box, and transcription is initiated when a protein complex called TFIID (which stands for transcription factor for RNA Polymerase II, D subunit) binds to it. TFIID is a multi-subunit machine, made of a TATA-binding protein (TBP) and TBP-associated factors (TAFs). Our lab has previously shown that an interaction between TAF1 and TAF7 inhibits TFIID's ability to initiate transcription of select genes. If TFIID cannot function properly, TATA-box controlled genes will be transcribed when they should be repressed. Some of these genes are cyclins, which are important for regulating the cell cycle. If they are misexpressed, a cell could proliferate with damaged DNA. This type of uncontrollable proliferation is a key component of cancers. Therefore, I am studying the interaction between TAF1 and TAF7 to determine the nature of TFIID inhibition. TAF7 is the proposed inhibitor in this model. I have cloned wild-type and mutant TAF7 genes, expressed and purified protein, and plan to use co-immunoprecipitation to determine if TAF1 and TAF7 associate. If the wild-type and mutant molecules have different affinities for TAF1, then I can conclude that the residue I've mutated is essential for TAF1 association. Later, I would like to study the difference in TAF1 activity in the presence of wild-type or mutant TAF7. This research asserts its relevance through the ubiquity of TFIID-mediated transcription regulation in every one of our cells, as well as the implication that TAF7 misexpression could lead to aberrant cell growth and possibly cancer.

POSTER SESSION 4

Commons East, Easel 47

4:00 PM to 6:00 PM

Effects of Ketogenic Metabolites on Neural Activity in Brain Slices of a Mouse Model of Dravet Syndrome

Kyuwoong Kim, Senior, Biology (General)
Mentor: Franck Kalume

Dravet Syndrome (DS) is a rare form of epilepsy characterized by severe seizure phenotype beginning in early childhood. It is primarily caused by loss-of-function mutation in SCN1A gene which encodes a voltage-gated sodium channel Nav1.1. DS is resistant to most commercially available anti-epileptic drugs. However, ketogenic diet (a high fat and low carb diet), has been shown to provide considerable seizure protection to patients with this epilepsy. Its mechanisms of action are not completely known. One of the hypotheses explaining the efficacy of ketogenic diet is that the elevated level

of ketone bodies leads to the modification of Tricarboxcylic Acid Cycle (Krebs Cycle), increase GABA (Gamma Amino Butyric Acid) synthesis, and promote energy production in brain tissues. This research study focused on understanding the effects of ketogenic diet on epiletiform activity in DS mice. We recorded field potentials from hippocampi of wild-type and DS mice in brain slice preparations. We added ketogenic diet metabolites to the Artificial Cerebrospinal Fluid (ACSF) and investigate their effects on brain activity.