26th Annual Joel G. Hardman Student-Invited Pharmacology Forum

Vanderbilt Pharmacology Through the Decades:
Over 80 Years of Impact
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Student Abstracts
Poster 1

An atypical sensor kinase controls intrinsic resistance to polymyxin B in uropathogenic *E. coli*

Erin Breland, Kirsten Guckes, Ellisa Zhang, Maria Hadjifrangiskou

Urinary tract infections, accounting for the majority of antibiotic prescriptions, are primarily caused by uropathogenic *E. coli* (UPEC). The latest report by the World Health Organization places carbapenem-resistant Enterobacteriaceae as the third most critical pathogen for which new therapies are required. In cases of carbapenem resistance, a last-resort class of antibiotics is colistin, for which bacteria acquire resistance by modifying their cell wall. We have shown that UPEC can mount intrinsic resistant to polymyxin B via the interaction of two signaling systems, PmrAB and QseBC. Two-component systems are prototypically comprised of a histidine kinase (sensor) and a response regulator (responder). We present evidence that the QseC kinase relies on reverse-phosphotransfer to catalyze the de-phosphorylation of its cognate partner QseB. The QseC kinase is conserved across several Gram-negative pathogens; its interaction with its cognate partner QseB is critical for maintaining pathogenic potential. Here, we demonstrate that QseC-mediated de-phosphorylation of QseB occurs via reverse-phosphotransfer, which is atypical for bacterial sensors. We also demonstrate that kinase-inactive QseC variants were unable to mediate a wild-type stimulus response, indicating that QseC is required for maintaining proper QseB-PmrB-PmrA interactions and that it can be targeted for the development of anti-virulence strategies.
Altered potassium homeostasis in glaucoma and significance for retinal ganglion cell health

Rachel A. Fischer, Cathryn R. Formichella MS, Franklin D. Echevarria PhD, Roman Lazarenko PhD, Qi Zhang PhD, Rebecca M. Sappington PhD

Retinal ganglion cell (RGC) degeneration is the cause of irreversible blindness for millions of individuals worldwide. Glaucomatous vision loss results from degeneration of RGC axons, which comprise the optic nerve. Glaucoma produces functional deficits in RGCs prior to structural degeneration. There is a potential therapeutic window after the onset of functional deficits, but prior to irreversible, physical loss of RGC axons in which axonopathy could be interrupted and further vision loss prevented. Our goal is to identify factors involved in the progression from functional deficits to structural RGC degeneration. Glaucoma patients experience vision loss in clusters across their visual field, rather than in a uniformed manner. Studies in animal models of glaucoma indicate that RGC axonopathy occurs in similar patterns. This suggests that external cues in the immediate milieu may play a role in propagating disease progression to neighboring RGCs. Early RGC axonopathy includes functional deficits in axon transport. Preliminary data from our lab indicates that RGC transport deficits are accompanied by electrophysiological impairments. One possible explanation for these electrophysiological impairments is a disruption in the K⁺ homeostasis in the retina. Müller glia are responsible for removing excess K⁺ from the extracellular space via K⁺ siphoning. Our preliminary data indicates that there is a significant disruption in the K⁺ homeostasis in glaucomatous retina. We found changes in expression of Kir (inward rectifying) and K2P (two-pore) K⁺ channels in RGCs and Müller glia, as well as altered ion flux through K⁺ channels in cells subjected to glaucoma-related stressors. We are testing the hypothesis that local changes in the K⁺ homeostasis induces electrophysiological impairment in RGCs and propagate this dysfunction to neighboring RGCs.
Poster 3

Chemical Genetics of Cation Chloride Cotransporter Activation and Oligomerization

Francis J. Prael III, Eric Delpire, C. David Weaver

An estimated 1 in 26 people are predicted to develop epilepsy at some point in their life. Epilepsy, a chronic neurological condition defined by recurrent seizures, is a tremendous burden on those afflicted with the disease. With nearly one-third of epilepsy patients being refectory to all treatments, novel approaches for the treatment of epilepsy are urgently needed. In the C. David Weaver laboratory, I work on discovering and developing chemical tools targeting transporters that are dysfunctional in epilepsy. We will use these chemical tools to evaluate the therapeutic potential of correcting dysfunction of these transporters in epilepsy and to further characterize fundamental characteristics of these proteins relevant to their dysfunctional state in epilepsy. If successful, our work could lay the foundation for novel approaches to the treatment of epilepsy.
Mono-functional elements of arrestin-3 interact with extracellular signal-regulated kinase 2 (ERK2)

Nicole A. Perry, Bridget Collins, Tamer S. Kaoud, Kevin N. Dalby, T.M. Iverson, and Vsevolod V. Gurevich

The arrestin proteins are canonically known for their role in the desensitization and internalization of G protein-coupled receptors (GPCRs); however, arrestin has also been implicated as a molecular scaffold for various effectors including clathrin, AP2, phosphodiesterases, and mitogen activated protein kinase (MAPK) cascades. Recent investigations have revealed that arrestin interacts with its binding partners via mono-functional elements, which are protein fragments that retain a single function of an intact protein. Our research identified peptide elements of the non-visual arrestin-3 that interact with extracellular signal-regulated kinase 2 (ERK2). ERK2 is the final effector in the Raf-Mek-ERK2 kinase cascade that regulates cell death. In addition to identifying the elements of arrestin-3 that interact with ERK2, our study uses these peptide fragments for co-crystallization trials to elucidate the structural basis of arrestin/effector interaction. This work will provide investigators with a clearer understanding of arrestin-mediated MAPK activation.
Modulation of synaptic transmission: G protein specificity of inhibitory adrenergic a2a receptor

Yun Young Yim, Katherine Betke, W. Hayes McDonald, Ralf Gilsbach, Yunjia Chen, Karren Hyde, Qin Wang, Lutz Hein, and Heidi Hamm

Modulation of neurotransmitter exocytosis by activated Gi/o-type G-protein coupled receptors (GPCRs) is a universal regulatory mechanism used both to avoid overstimulation and to influence circuitry. One of the known modulation mechanisms is Gbg and soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex interaction. There are 5 Gb and 12 Gg subunits, but their protein level and subcellular localization, and whether specific Gbggs are activated by a given GPCR in vivo are not known. Presynaptic a2a-adrenergic receptors (a2a-ARs) in both adrenergic (auto a2a-ARs) and non-adrenergic neurons (hetero a2a-ARs) inhibit neurotransmitter release and affect anesthetic sparing and working memory enhancement. Here, we examine whether auto a2a-ARs in sympathetic neurons use the same Gbg dimer as hetero a2a-ARs in other neuronal types and that particular Gbg dimer is used to inhibit exocytosis by interacting with SNARE. So far, we find Gβ2, Gγ2, and Gγ3 preferentially interacting with activated auto a2a-ARs. We also see a basal Gbg-SNARE interaction. This basal interaction is enhanced by 2 fold upon the auto a2a-ARs activation. Further understanding Gbg specificity and Gbg-SNARE interaction may offer new insights into the normal functioning of the brain, as well as better understanding of disease progression.
Poster 6

Essential Role of HIPK2 in Maintaining Basal Cardiac Homeostasis

Yuanjun Guo, Jennifer Sui, Qinkun Zhang, Thomas Force, Hind Lal

Heart failure is the leading cause of mortality and morbidity worldwide. Despite progresses made in the treatment and management of most cardiovascular diseases, the prevalence and mortality of heart failure are still rising. Thus, new targets for heart failure treatment are in desperate need. Based on an integrated transcriptional analysis, we identified a previously unknown target—Homeodomain-interacting protein kinase 2 (HIPK2). HIPK2 is a conserved nuclear serine-threonine kinase with a well-established role in cancer biology. However, the role of HIPK2 in myocardial biology is unknown. To determine the role of HIPK2 in myocardial biology, we created αMHC-Cre driven cardiomyocyte-specific HIPK2 knockout mice. We found that the heterozygous HIPK2 knockout mice developed heart failure at the age of 6 months, as reflected by significantly reduced left ventricle fractional shortening and ejection fraction. In the αMHC-MerCreMer knockout mice, the heart function of knockout mice were also significantly decreased after HIPK2 deletion induced by Tamoxifen. These findings suggest that HIPK2 in cardiac myocyte is required to maintain normal cardiac homeostasis and its deletion leads to marked cardiac dysfunction. In the future study, we plan to delineate the underlying molecular mechanism by which HIPK2 deficient hearts leads to severe cardiac dysfunction.
Prescription opioid abuse is a nationwide epidemic. Opioids induce a rewarding release of dopamine from dopaminergic (DA) neurons in the ventral tegmental area (VTA) of the brain. These neurons express G protein-gated inwardly-rectifying potassium channels (GIRKs), and, while GIRK channels are composed of GIRK1, 2, 3, and/or 4 subunits that assemble into homo- and heterotetramers, the VTA DA neurons express only non-GIRK1-containing GIRK channels. These GIRK channels are implicated in the regulation of substance abuse. To further investigate this role of GIRKs, we sought to determine if pharmacological modulation of GIRKs in the VTA can affect behavior. However, non-GIRK1-subunit-containing selective GIRK channel modulators do not exist. Using a high-throughput thallium-flux assay, we screened a library of >110,000 compounds using GIRK2-overexpressing HEK293 cells. We discovered that the natural product ivermectin activated GIRK2 channels. We found that ivermectin also activated GIRK1/2 and GIRK1/4 channels. To allow us to pharmacologically probe DA neurons in the VTA, future studies will explore analogs in search of molecules that are potent, efficacious, and selectively activate non-GIRK1-containing channels. Further, compounds will be studied using in vitro and ex vivo electrophysiology, and we will assess the efficacy of compounds in rodent behavioral models of craving and addiction.
Poster 8

Investigation of Lamin A/C Cardiomyopathy Using Patient Derived Induced Pluripotent Derived Stem Cells

Shan Parikh, Kevin Bersell, Lili Wang, Marcia Blair, Andrea Murad, Quinn Wells, Bjorn Knollmann

Lamin A/C (LMNA) mutations are the second most common cause of familial dilated cardiomyopathy (DCM). LMNA encodes nuclear lamins which are intermediate filaments of the inner nuclear membrane. Patients with LMNA DCM present with early signs of arrhythmias and often display a rapid deterioration in cardiac function. Previous induced pluripotent derived stem cell (iPSC) modeling of cardiac laminopathy did not demonstrate defects in cardiomyocyte (CM) contractility nor provide evidence of arrhythmogenesis. Developing a robust human model that recapitulates disease manifestations of LMNA cardiomyopathy is a critical step for understanding CM-intrinsic defects contributing to disease pathogenesis. As such, our lab hypothesized that iPSC-CMs derived from a laminopathy patient would recapitulate the contractile dysfunction of the disease if studied in monolayer using flexible matrigel substrate.

Patients with symptomology indicative of LMNA DCM were recruited for clinical testing. Peripheral blood mononuclear cells were isolated and reprogrammed into iPSCs. CMs were generated using a chemical differentiation method and then subjected to molecular and functional assessment. Cardioexcyte96 based detection of impedance was utilized for analysis of contractility of iPSC-CMs in a monolayer. We identified a large pedigree with DCM in which affected individuals had a putatively pathogenic mutation within LMNA. Our results are the first to provide evidence for contractile dysfunction in an iPSC-CM model of cardiac laminopathy.
Previous studies showed decreased intestinal N-acylphosphatidylethanolamines (NAPE) biosynthesis in animals on high fat diet (HFD). Administration of recombinant *E. coli* Nissle 1917 (EcN) expressing a NAPE acyltransferase gene (pNAPE-EcN) generate NAPEs and reduce food intake. NAPEs may reduce food intake by increasing the sensitivity to short term (CCK) and long term (Leptin) regulators of food intake. To test if pNAPE-EcN increased sensitivity to leptin, mice were fed a high-fat diet (HFD) (4 weeks); then given vehicle (0.125% Gelatin), control bacteria (pEcN) or pNAPE-EcN (4 weeks); then osmotic pumps implanted to administer either vehicle (HBSS) or leptin (5mg/kg, 1 week). When combined all leptin treated mice had significantly reduced 7 day food intake and increased weight loss. Mice receiving pNAPE-EcN had no greater reductions in food intake or body weight. Enhanced CCK sensitivity by pNAPE-ECN was tested by prefeeding mice with low fat diet for 10 days; followed by 2 weeks of vehicle, pEcN, or pNAPE-EcN treatment on HFD; then fasted (12 h), and injected with CCK (20 μg/kg) 30 min prior to restoring food. Food intake was measured (16 h). Saline injections did not significantly reduce 16 h food intake regardless of treatment. CCK injections in mice previously treated with pNAPE-EcN significantly reduce 16 h food intake compared controls. Treatment with pNAPE-EcN reduces food intake on a HDF in part by increasing sensitivity to CCK.
The antidepressant sensitive serotonin (5-HT) transporter (SERT, Slc6a4) affords powerful control over 5-HT signaling via rapid clearance of 5-HT from the extracellular space. Recently our lab identified a gain-of-function, autism-associated coding variant, SERT Gly56Ala. We developed a SERT Ala56 knock-in mouse model which exhibit increases in CNS 5-HT clearance and elevated 5-HT in whole blood, or hyperserotonemia, a well characterized biomarker in a sub-population of patients with ASD. SERT Ala56 mice also exhibit ASD like behaviors, such as decreased social interactions, decreased ultra-sonic vocalizations and repetitive like behaviors. Serotonin signaling was also altered in the SERT Ala56 mice as assessed by hypersensitive 5-HT1A and 5-HT2A receptors. SERT protein in Ala56 mice exhibit hyperphosphorylation which can be normalized by inhibition of p38 MAPK. Previous work has revealed p38α MAPK is the primary isoform responsible for SERT regulation. We hypothesize that p38α MAPK shifts the population of transporter into an outward facing conformation state that is dictated by protein-protein interactions. Using candidate and proteomic-based approached, we are assessing differences between WT and SERT Ala56 interacting proteins. We also hypothesized that selective, CNS penetrant, p38α MAPK inhibitors such as MW108 or MW150 might attenuate the ASD-like phenotypes in the SERT Ala56 model. Treatment with MW108 (10mg/kg, QD x 1 week) or MW150 (5 mg/kg, QD x 1 week) normalized SERT Ala56-mediated hypersensitivity of 5-HT1A and 5-HT2A receptors and increases in hippocampal 5-HT clearance. Chronic MW108 or MW150 treatment also normalized SERT Ala56-mediated social deficits. Moreover, genetic elimination of p38α MAPK in 5-HT neurons of SERT Ala56 mice resulted in the normalization of 5-HT2A receptor hypersensitivity and social deficits. These are the first studies to provide pharmacologic and genetic evidence that targeting p38α MAPK signaling may be a potential treatment of ASD.
The Conn Laboratory

Fisher NM, James MT, Niswender CM, and Conn PJ

The Conn lab works closely with other groups within the Vanderbilt Center for Neuroscience Drug Discovery to study two classes of G-protein coupled receptors: the metabotropic glutamate receptors and the muscarinic acetylcholine receptors. Projects in the lab aim to study the role of these receptors in brain physiology and behavior, and to validate their potential as novel therapeutic targets for a range of neurological and psychiatric disorders. We use newly developed small molecule allosteric modulators to determine the roles of individual receptor subtypes. James Maksymetz and Nicole Fisher are current pharmacology graduate students within the Conn lab. James studies M1 muscarinic receptor-mediated long term depression and neuromodulation in the prefrontal cortex using brain slice electrophysiology, which has implications for the treatment of schizophrenia. Nicole is co-mentored by Colleen Niswender and her project focuses on validation of mGlu7 as a novel target for Rett syndrome.
Utilization of a processing algorithm for the identification of a clinical resistant hypertension population from electronic medical records

Megan M. Shuey, Nancy J. Brown, Joshua C. Denny, Todd L. Edwards

Resistant hypertension is a common clinical problem with an estimated prevalence of 8.4 to 17.4% of the hypertensive population. These individuals have uncontrolled blood pressure despite concurrent use of three medications, including a diuretic, and are at an elevated risk of developing organ damage, including myocardial infarction, stroke and heart and renal failure. We have developed an algorithm that identifies patients within a clinical population with resistant hypertension.

Using Vanderbilt’s BioVU, a DNA databank containing DNA samples from clinical patients and their linked electronic medical records, we identified 55,477 subjects with an incidence of a hypertension associated ICD9 codes. Our algorithm queries all subjects within BioVU for identification of patients meeting the diagnostic criteria for resistant hypertension and easily controlled hypertension. The identified resistant hypertensive population represents 14.18% (7,865 out of 55,477) of all individuals with an incidence of hypertension as indicated by ICD9 code within BioVU. An incidence of 14.18% matches the incidence values identified in previous epidemiologic and clinical trial studies lending support to our algorithm’s efficacy. A blind chart review revealed the algorithm’s call accuracy to be 94.67% (142 of 150).

The accuracy of algorithm call, characteristics of the case and control populations, as well as congruence with estimated incidence rate lend support for our algorithm’s ability to accurately identify subjects with resistant hypertension from a clinical population utilizing electronic medical record data.
Poster 13

Two Interconnected Two-Component Systems in Uropathogenic *Escherichia coli* sense Acid and Pyruvate

Bradley Steiner, Allison Eberly, Ellisa Zhang, and Maria Hadjifrangiskou, Ph. D.

Bacterial two-component systems (TCSs) control many bacterial responses to environmental change via the activation of a membrane-embedded sensor kinase, which has molecular specificity for a cognate response regulator protein. While non-cognate partner interactions among TCSs are rare, we have recently demonstrated that closely related TCSs can physiologically interact under specific conditions, imparting a fitness advantage to bacterial pathogens. In this study we describe another example of inter-connectivity for the metabolite-sensing TCSs YpdAB and YehUT. In K12 *E. coli* YpdAB and YehUT respond to changes in serine and pyruvate levels and alter the expression of putative transporter genes *yhjX* and *yjiY*. *YhjX* and *yjiY* are upregulated in uropathogenic *E. coli* (UPEC) during acute urinary tract infection. Here we show that pyruvate induced expression of UPEC *yhjX* and *yjiY* requires components of both YpdAB and YehUT to maintain proper transcriptional activity and fidelity of response. In addition, we report that acidification of growth media also activates downstream target gene expression in a similar manner, identifying another stimulus for this system.
Docking and priming of secretory vesicles at the plasma membrane is a key step in regulated exocytosis and is modulated by a multitude of lipid and protein interactions. Calcium-dependent activator protein for secretion 1, CAPS1, is a CNS and endocrine-specific protein that mediates docking and priming primarily through interactions of its Munc-homology domain with the SNARE protein syntaxin-1. Ablation of the protein decreases the size of the readily releasable pool of vesicles which reduces the amount of chemical message released from cells. CAPS1 transcripts undergo adenosine-to-inosine RNA editing which results in a glutamate to glycine substitution in the C terminal domain. Recent work performed in our lab has demonstrated significant functional consequences of the A-to-I editing event, including differences in the vesicle release rate and mode of exocytosis. To further investigate the physiological role of CAPS1 RNA editing, we will use non-edited and edited CAPS1 mouse models to study exocytosis events using microdialysis, and SynpHTomato and Q dot tracking in primary neurons. Additionally, we will study CAPS1’s interactions with vesicle membrane proteins to discern the differences in vesicle binding in relation to editing status. These studies will provide further insight into the physiologic role of CAPS1 RNA editing and will investigate the molecular mechanism by which RNA editing modulates CAPS1’s functions.
Poster 15

A lipid kinase, IPMK, as a potential regulator of the NR5A nuclear receptors

Mark Crowder and Ray Blind

It is established that the phosphatase and tumor suppressor PTEN (phosphatase and tensin homolog deleted on chromosome 10) is mutated in a variety of cancers, often resulting in PTEN loss of function. At cell membranes, PTEN opposes the activity of phosphatidylinositol 3-kinases (PI3Ks), which generate the signaling lipid PIP3. PI3K activation leads to the stabilization of key cell signaling complexes at the membrane via PH-domain mediated interactions with PIP3. This complex formation is required for signal transduction to occur; however, upregulated PI3K activity (overproduction of PIP3) is associated with oncogenesis. However, drugs targeting membrane PI3Ks have widely failed. Our lab studies a nuclear PI3K called IPMK (inositol polyphosphate multikinase) that converts PIP2 to PIP3 while bound to a transcription factor, NR5A1. IPMK conversion of PIP2 to PIP3 while bound to NR5A1 increases the expression of NR5A1 target genes. PTEN, in an opposing manner, is able to dephosphorylate PIP3 to PIP2, causing repression of NR5A1 target genes. I hypothesize that an NR5A1 homolog, NR5A2, is also regulated by IPMK and that PIP3 stabilizes transcriptional complexes via PH-domain mediated interactions similar to its role at cell membranes. If proven, these results would implicate IPMK as a cancer drug target.
Following a severe injury such as a burn, patients undergo a systemic response, termed the acute phase response, in order to withstand the trauma and heal damaged tissues. During the first hours to days of the acute phase response, the body is fighting blood loss and infection in order to survive. Approximately 2-3 days after a severe injury, repair of the injured tissues begins. Decades ago, patients died during the “survival phase” due to blood loss and infection, but with the development of antibiotics and blood transfusions, these patients survive and often experience the challenge of poor tissue repair. It has been clinically observed that many regenerative factors are consumed during the “survival phase”, contributing to poor tissue repair later on. In order to treat severe injury patients, key systemic changes in regenerative factors that occur immediately following severe injury must be identified and targeted to improve tissue repair.

The protease plasmin is a key regenerative protease and mediator of the switch between survival and tissue repair modes within the acute phase response. We have determined that following a severe burn injury in both mice and humans, plasmin is rapidly activated and degraded, resulting in a >50% depletion in circulating plasmin precursor, plasminogen. This loss of plasmin occurs within the critical initiation of tissue repair and lasts approximately 7 days. It has been demonstrated that plasmin is essential for repair of skin, muscle, and bone, suggesting that a loss of plasmin following burn presents a potential risk for impaired healing. Using a mouse model of burn with concurrent muscle injury, we have demonstrated that burn-induced loss of plasmin results in calcification and impaired healing of injured muscle. Pharmacologic enhancement of plasmin activity restored muscle regeneration in mice following a severe burn. The results of this study suggest that the loss of plasmin is a pathologic systemic change following a severe burn injury that potentially may be targeted therapeutically in order to improve tissue repair in burn patients.
Dopamine (DA) plays an important role in the central nervous system by regulating a variety of functions, including cognition and motivation. While DA dysfunction is known to be involved in several neuropsychiatric disorders its role in autism spectrum disorder (ASD) is largely unknown. We have recently identified several single nucleotide variants in the SLC6A3 gene in individuals with ASD. The SLC6A3 gene encodes the DA transporter (DAT), a presynaptic membrane protein critical to DA neurotransmission. Specifically, DAT mediates the active high affinity re-uptake of DA from the synapse into the synaptic bouton, maintaining DA homeostasis. We report a novel SLC6A3 variant identified in an ASD proband from whole exome sequencing. This variant encodes an in-frame deletion of three nucleotides, resulting in the deletion of an asparagine at position 336 (∆N336). In vitro experiments demonstrate that ∆N336 results in nearly absent DAT-dependent DA uptake despite normal surface expression. We utilize Drosophila melanogaster as an animal model to determine functionally and behaviorally whether expression of hDAT∆N336 in DA neurons results in DA dysfunction.

Drosophila has enabled rapid progress in neuroscience research due to its cost-effectiveness, genetic tractability, rapid life cycle and conserved mechanisms of neurotransmission. Notably, mechanisms mediating DA neurotransmission including transport, synthesis, and secretion are largely conserved. Expression of ∆N336 in Drosophila leads to impaired brain DA uptake and reverse transport of DA. Moreover, flies expressing hDAT ∆N336 display increased basal locomotion and grooming. This data is consistent with increased levels of extracellular DA. We evaluated further whether impairments in DA clearance affect defensive responses (i.e. freezing or fleeing) and/or social interactions, behaviors which are altered in individuals with ASD. Flies expressing ∆N336 display aberrant freezing, fleeing, as well as, social behaviors. These results add to the growing body of literature associating altered regulation of DA homeostasis to complications in ASD.
Poster 18

Effects of secretase inhibitors on amyloid precursor protein trafficking

Claire E. DelBove, Claire E. Strothman, Qi Zhang

Amyloid precursor protein (APP) is cleaved by three enzymes known as the α-, β- and γ-secretases to produce a variety of physiologically important cleavage products. Amyloid beta (Aβ), which contributes to Alzheimer’s disease pathogenesis, is produced from sequential cleavage by β-secretase and γ-secretase. It is not well understood how γ-secretase inhibition alters the fate of full-length APP, which may cause unexpected consequences. To directly explore these questions in live neurons, we conducted live-cell fluorescence imaging in conjunction with a set of biochemical measurements. Using immunocytochemistry and time-lapse live cell imaging, we confirmed that the fluorescent APP is localized and trafficked in a manner consistent with endogenous APP. An examination of the effects of secretase inhibitors revealed that application of α- and β-secretase inhibitors reduce the surface fraction of APP, application of γ-secretase inhibitor increases the surface fraction of APP and there is an unexpected interaction when all three secretases are inhibited that further increases the surface fraction of APP. An increased mechanistic understanding of APP distribution and trafficking among different subcellular locations and between major secretase pathways is essential for designing effective therapeutic strategies to prevent Aβ accumulation and neurodegeneration.
Poster 19

Decreased mtDNA decreases mitochondrial creatine kinase: Energetic instability in L-DOPA Induced Dyskinesia

Warren, EB and Konradi, C

L-DOPA Induced Dyskinesia (LID), characterized by excessive and uncontrolled movements, occurs in most Parkinson's Disease patients treated with L-DOPA, but some patients develop LID more quickly than others. One of the factors differentiating dyskinetic PD patients from nondyskinetic patients is a reduction of mtDNA in the striatum. We hypothesize that this decreased mtDNA in the putamen of dyskinetic patients plays a critical role in accelerating the development of LID by destabilizing the striatum's energetic capacity. We have established a model of this mtDNA reduction by treating primary striatal cultures with ethidium bromide (EtBr), which at low concentrations accumulates in mitochondria and selectively reduces mtDNA while sparing nuclear DNA. EtBr-depleted mtDNA induces a dramatic reduction in mtRNA, which corresponds to a decrease in mitochondrial respiratory activity. Moreover, decreased mtDNA in these cultures results in a neuron-specific reduction in the expression of mitochondrial creatine kinase (mtCK), an enzyme crucial for shuttling phosphate from mitochondrially-produced ATP to sites of high energy demand. Expression of mtCK was also significantly reduced in dyskinetic PD patients. This reduction may point to a relationship between mtDNA quantity and an ability to appropriately respond to high energy stimuli.
Research in our laboratory seeks to fuse computational and experimental efforts to investigate proteins, the fundamental molecules of biology, and their interactions with small molecule substrates, therapeutics, or probes. We develop computational methods with three major ambitions in mind.

A) To enable protein structure elucidation of membrane proteins the primary target of most therapeutics and large macromolecular complexes such as viruses;
B) Design proteins with novel structure and/or function to explore novel approaches to protein therapeutics and deepen our understanding of protein folding pathways.
C) Understand the relation between chemical structure and biological activity quantitatively in order to design more efficient and more specific drugs.

Crucial for our success is the experimental validation of our computational approaches which we pursue in our laboratory or in collaboration with other scientists.

Current research applications focus on new approaches to a) drug and probe development for neurodegenerative disorders and diseases including Schizophrenia, Alzheimer's, and Parkinson's, b) understanding the structural determinants of antidepressant binding to neurotransmitter transporters, c) cardiac arrhythmia as caused by the complex interplay of potassium channel regulation and drug interactions, d) multidrug resistance in cancer and bacterial cells related to multidrug transporter proteins, and e) structural basis of viral infections and antibody activity.
Poster 21

Potassium Channel Pharmacology and Physiology - Denton Laboratory

Jerod Denton, Sujay Kharade, Eric Figueroa, and Nathan Winters

The major focus of the Denton laboratory is on the development and use of small-molecule tool and pre-therapeutic compounds for investigating the structure, integrative physiology, and “druggability” of inward rectifier potassium (Kir) channels. Studies of knockout mice and human diseases have suggested that some Kir channel family members represent unexploited drug targets for diseases such as hypertension, cardiac arrhythmias, and pain. With few exceptions, however, the molecular pharmacology of the Kir family has remained undeveloped, miring progress toward assessing their therapeutic value. To overcome this barrier, our group employs leading-edge technologies that include high-throughput screening, medicinal chemistry, molecular biology, and voltage-clamp electrophysiology to develop a versatile ‘toolkit’ of selective Kir channel modulators.
Poloxamer 188 Protects Isolated Cardiomyocytes from Hypoxia/Reoxygenation Injury

Michele M. Salzman, BS; Benjamin J. Hackel, PhD; Frank S. Bates, ScD; Jason A. Bartos, MD, PhD; Demetris Yannopoulos, MD; Matthias L. Riess, MD, PhD

Introduction:
Many patients who experience cardiac arrest still die or suffer cardiac damage, even when the best CPR is delivered. This is due to the fact that the reintroduction of blood flow at the start of CPR causes additional injury. Determining the mechanism of a compound administered during reperfusion on cardiomyocyte dysfunction caused by ischemia/reperfusion (I/R) injury could improve clinical practices in the future.

Hypothesis:
P188, with its unique chemical properties, protects cardiomyocytes against H/R injury.

Methods:
Cardiomyocytes underwent H/R ± P188 or polyethylene glycol (PEG). Endpoints were markers of cell number, cell injury (lactate dehydrogenase [LDH] release), and intracellular Ca2+.

Results:
Cell number significantly decreased, and LDH release and [Ca2+]i significantly increased, following H/R. P188 present during reoxygenation significantly increased cell number and significantly decreased Ca2+ influx in the H/R conditions. Only 1mM P188 significantly decreased LDH release in the H/R conditions; the other P188 concentrations exhibited a non-significant trend towards decreased LDH release. No PEG concentration had an effect.

Conclusions:
P188, administered during reoxygenation, protected cardiomyocytes from H/R injury by improving cell number, reducing Ca2+ influx, and decreasing LDH release. These results support a possible protective role of P188 against I/R injury when it is administered during reperfusion.
The phosphoinositide 3-kinases (PI3Ks) have well established oncogenic membrane signaling functions, working in concert with PTEN to regulate PI(3,4,5)P3 (PIP3) in cytoplasmic membranes. However, the functions PI3K enzymes serve in the nucleus remain understudied, despite recent advances in our understanding of the roles nuclear PTEN plays in oncogenesis. Inositol polyphosphate multikinase (IPMK) is an atypical nuclear PI3K enzyme, which plays critical roles in transcriptional regulation, nutrient sensing, and cell death. We have shown IPMK antagonizes a nuclear PTEN activity decoupled from other known PI3K enzymes: IPMK directly phosphorylates PI(4,5)P2 (PIP2) bound to the nuclear receptor SF-1 activating SF-1 target genes. A major obstacle to characterizing IPMK has been the lack of a stable expression construct as several labs have failed to purify IPMK at sufficient quantitates from both bacterial and mammalian sources for experiments other than kinetic studies. To solve this problem, I have successfully designed a stable human IPMK construct via protein engineering. We are utilizing our synthetic IPMK to construct an atomic resolution model of IPMK, determine IPMK phosphorylation sites in response to upstream stimuli, and novel substrates of IPMK. Together, these studies will provide a broad framework of how IPMK functions on the molecular level.
Malignant migrating partial seizures of infancy (MMPSI) is a rare, severe form of epilepsy that begins in early childhood. Due to the severity, and number of seizures, which can range from five to 30 a day, affected individuals can suffer profound developmental delays and intellectual impairment. The majority of MMPSI cases are considered pharmacoresistant causing affected individuals to not survive past early childhood. At least 20 de novo mutations affecting three functional domains of the potassium (K⁺) channel Slack have been reported in patients with MMPSI, making it the most frequent genetic cause of MMPSI. Slack is a sodium-activated K⁺, which is encoded for by KCNT1, is found within inhibitory interneurons. Its activity contributes to the post-action potential slow hyperpolarization allowing for repetitive firing. Gain-of-function mutations have been reported within the pore-forming, regulator of conductance of K⁺, and NAD⁺ binding domains of the channel. It has been proposed that these mutations cause prolonged hyperpolarization resulting in an imbalance between neuronal excitation and inhibition leading to impaired inhibitory neuronal function and seizures. There is currently an unmet need to identify more selective drugs for KCNT1 induced epilepsies. Discovery and characterization of such compounds may provide a foundation for developing new clinical tools for the treatment of MMPSI.
Functional consequences of RNA editing on mGlu₄ dimerization

Christopher S. Hofmann, Colleen M. Niswender and Ronald B. Emeson

mGlu₄, a member of the group III metabotropic glutamate receptors, functions as a presynaptic autoreceptor controlling synaptic neurotransmitter release. Its pre-mRNA transcripts are modified by adenosine-to-inosine (A-to-I) RNA editing events at up to 5 putative sites, resulting in amino acid substitutions along the proposed interface of mGlu receptor dimers. While mGlu₄/mGlu₂ heterodimers exist, it is unknown whether RNA editing affects mGlu₄ homo- or heterodimerization. Profiles of mGlu₄ editing patterns in multiple brain regions and peripheral tissues will be quantified using multiplexed Illumina sequencing. The extended duplexes required for editing will be identified using the folding algorithm, mfold, and validated using an in vitro editing assay by introduction of duplex-disrupting mutations to ablate A-to-I conversion, accompanied by compensatory mutations that restore both duplex structure and site-selective editing. Transcript and receptor isoform stability will be assessed in HEK293 cells using quantitative RT-PCR and Western blotting strategies. Potential changes in ligand-binding and signaling properties will be quantified using saturation binding and assays for inhibition of the cAMP cascade. Surface trafficking of receptor isoforms will be assessed using surface biotinylation approaches, while heterodimerization of mGlu₄/mGlu₂ will be determined using thallium flux assays in the presence of positive allosteric modulators that potentiate signaling in mGlu₄ homodimers and mGlu₄/mGlu₂ heterodimers or only potentiate signaling in mGlu₄ homodimers.
FUNCTIONAL SIGNIFICANCE OF A TRUNCATED SEROTONIN 2C RECEPTOR

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5HT2C transcripts are alternatively spliced to generate at least two additional splice variants that encode truncated receptors. Both truncated isoforms of the receptor have been shown to lack serotonergic ligand binding and phosphoinositide (PI) hydrolysis activity. One of these truncated splice variants (5HT2C-TR) is a highly abundant transcript, accounting for 30-60% of total 5HT2C mRNAs in discrete brain regions. Recent studies using heterologous expression systems have shown that expression of 5HT2C-TR decreases specific radioligand binding to the full-length 5HT2C receptor (5HT2C-FL) via sequestration of heterodimers in the endoplasmic reticulum, a phenotype that has been further validated through confocal imaging of GFP/YFP-tagged fusion receptors. We have also confirmed that co-expression of various edited 5HT2C-FL isoforms with 5HT2C-TR in heterologous cells causes a decrease in basal and agonist-stimulated PI hydrolysis, thus demonstrating a physiological consequence for this ER retention phenotype. Many in vitro studies have shown a similar dominant-negative function for highly truncated GPCRs; however, these findings have not been validated in vivo. To this end, we have developed and are currently characterizing an inducible mouse model that will enable overexpression of 5HT2C-TR. Our findings may identify the 5HT2C-FL/5HT2C-TR heterodimer as a novel pharmacological target.
Stress contributes to the vulnerability for relapse to drug-seeking behavior. Therefore, the beta adrenergic receptor (β-AR) antagonist propranolol, which attenuates physiological responses to stress, has been investigated as a potential therapy against relapse. While propranolol blocks stress-induced reinstatement of cocaine seeking in rodents, the β2-AR agonist clenbuterol reinstates seeking. The bed nucleus of the stria terminalis (BNST) is a region that is critical for the actions of β-AR ligands on reinstatement. However, how β-ARs regulate BNST activity during basal and stressful conditions remains poorly understood. To determine if β2-AR activation is sufficient to increase BNST activity, brain slices containing the BNST were acutely prepared from c-fos-EGFP transgenic mice, in which a c-fos-enhanced green fluorescent protein (EGFP) fusion protein is driven by the c-fos promoter. The slices were treated with either vehicle or 10 μM clenbuterol. We found that clenbuterol induces a robust c-fos response within dorsal BNST neurons, but not in the ventral BNST. These findings demonstrate that β2-ARs can regulate BNST function by increasing the activity of intrinsic neuronal populations independent of circuit activity outside of the BNST. To determine if β-ARs are necessary for stress-induced changes in BNST activity, mice were treated with propranolol and subjected to 60 minutes of restraint stress prior to slice preparation. We found that propranolol prevented stress-induced expression of c-fos in the BNST, suggesting that β-ARs are necessary for stress-induced regulation of BNST function. In future studies, we will perform experiments to test the mechanism underlying clenbuterol-induced changes in c-fos expression, as well as perform experiments to determine the population of BNST cells that are regulated in this manner. These studies will provide insight into β2-AR receptor activity in the BNST and will inform future studies looking at the role of these receptors in stress-induced reinstatement of drug-seeking behavior.
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Progress toward the Development of Novel, Selective Positive Allosteric Modulators (PAMs) for the Metabotropic Glutamate Receptor Subtype 7 (mGlu$_7$)

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The metabotropic glutamate receptor 7 (mGlu$_7$) is one of eight subtypes of metabotropic glutamate receptors in the body. mGlu$_7$ is widely expressed throughout the brain—specifically, on the presynaptic terminals of neurons. There, mGlu$_7$ is believed to act as a synaptic regulator to prevent overstimulation, reducing the amount of glutamate and GABA released from the synapse upon its activation. This function of mGlu$_7$ is important for the maintenance of proper neurotransmission. For example, polymorphisms in the gene GRM7, which encodes mGlu$_7$, have been linked to many different neurological disorders including anxiety disorder, depression, schizophrenia, epilepsy, and autism.

The biology of metabotropic glutamate receptors (mGlu$_4$, mGlu$_8$), including mGlu$_7$, have been probed in these disorders, but the specific study of mGlu$_7$’s role has been difficult, in part, due to a lack of specific tool compounds. To date, there have been no fully selective mGlu$_7$ positive allosteric modulators with favorable pharmacokinetic properties available for in vivo studies. Currently available tool compounds are non-selective and/or metabolized rapidly making them unamenable for animal studies.

My project aims to develop and validate a selective, brain-penetrant, mGlu$_7$ PAM with favorable pharmacokinetic properties for in vivo proof-of-concept studies modeling neurological disorders.
Social interaction deficits and anxiety are highly comorbid symptoms in several psychiatric illnesses including autism spectrum disorder (ASD). Two brain regions that are involved in the regulation of these two behaviors are the basolateral amygdala (BLA), a well-established regulator in the development of anxiety and social interaction, and the nucleus accumbens (NAc), a critical region for regulation of reward and motivation. We have used in vivo optogenetics to assay the role of the BLA-NAc circuit in social interaction and anxiety-like behaviors in a mouse model. Our research demonstrates that activation of this pathway at 20hz and 5hz stimulation patterns causes a decrease in social interaction, as assayed by the three chamber social interaction task, and no effect on anxiety-like behavior. Additionally, our data show the glutamatergic BLA-NAc circuit is sensitive to endogenous cannabinoid (eCB) signaling. These findings could be relevant for elucidating pathophysiological mechanisms and therapeutic approaches for the treatment of psychiatric disorders characterized by increased anxiety and social dysfunction including ASD.
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Traumatic Skeletal Muscle Calcification: A Balance of Pyrophosphate and Inorganic Phosphate

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Introduction: Trauma-induced skeletal muscle calcification is a spectrum of disease, including dystrophic calcification and heterotopic ossification (HO), that may occur following severe injuries such as burn, blast, neurologic and musculoskeletal injuries, as well as certain orthopaedic procedures. Under normal physiologic circumstances, when the body undergoes injury, innate protection mechanisms inhibit the formation of calcification within skeletal muscle. Pyrophosphate is a well described anti-mineralization molecule that has been demonstrated to protect skin, the heart, and the kidneys from aberrant mineralization. Through this work, we will investigate the role of pyrophosphate plays in protecting skeletal muscle from mineralization following injury with the hypothesis that the balance of pyrophosphate to inorganic phosphate is critical for limiting mineralization in soft tissues.

Results: Using our validated model of trauma-induced skeletal muscle calcification in combination with either 1) a pyrophosphate deficiency or 2) a high phosphate diet, we demonstrated that the balance of pyrophosphate to inorganic phosphate is critical such that either a loss of pyrophosphate or an enhancement of inorganic phosphate through diet is sufficient to form skeletal muscle calcification following injury. Next, given our findings, we aimed to test our previously successful therapeutic method of enhancing plasmin activity to prevent the initial formation of skeletal muscle calcification in both mouse models. Starting at the time of weening, male mice were fed a high phosphate diet and were then administered an antisense oligonucleotide (ASO) specific for α2antiplasmin or a non-targeting control (100mg/kg/week) beginning two weeks prior to injury and continuing weekly. Following α2antiplasmin ASO treatment, we observed a marked decrease the development of skeletal muscle calcification in both models following injury at 7 days following injury as compared to control treated animals (P=<0.001). Therefore, these results distinguish plasmin activity as a promising therapeutic target for mitigating the development of skeletal muscle calcification following injury in a plasmin deficiency-independent model.

Discussion: Through this work, we have demonstrated that skeletal muscle calcification following injury is multifactorial. As such, alteration to multiple pathways, either genetic or environmental, can predispose skeletal muscle to calcification. Conversely, we have demonstrated that enhancement of just the fibrinolytic pathway, by targeting plasmin activity, is sufficient to provide cross-protection against high phosphate diet-induced skeletal muscle calcification following injury.

Significance: Since plasmin also supports bone homeostasis and fracture repair, increasing plasmin activity represents the first pharmacologic strategy to prevent skeletal muscle calcification without adversely affecting systemic bone physiology or concurrent skeletal muscle and bone regeneration. Therefore, these findings support further therapeutic development and potential bench to bedside translation.