NIH SHORT-TERM RESEARCH TRAINING FOR MINORITY STUDENTS

SYMPOSIUM PROGRAM & ABSTRACTS
HEALTH PROFESSIONS, NURSING, PHARMACY (HPNP) BUILDING
ROOM G-316

UF | UNIVERSITY of FLORIDA

College of Medicine

July 28, 2011
9:00 A.M.

University of Florida College of Medicine
Office of Minority Affairs

NIH SHORT-TERM RESEARCH TRAINING FOR MINORITY STUDENTS

The program's success is due largely to the active participation of the faculty mentors listed below:

Christine Baylis, Ph.D.	Department of Physiology & Functional Genomics
Adriaan Bruijnzeel, Ph.D.	Department of Psychiatry
Kirk Conrad, MD	Department of Physiology & Functional Genomics
Judy Delp, Ph.D.	Department of Physiology & Functional Genomics
Dorette Ellis, Ph.D.	Department of Pharmacodynamics
Reginald Frye, Ph.D.	Department of Pharmacotherapy and Translational Research
Daniel Hahn, Ph.D.	Department of Entomology and Nematology
Hideko Kasahara, Ph.D.	Department of Physiology & Functional Genomics
Clayton Matthews, Ph.D.	Department of Pathology and Laboratory Medicine
Barry Setlow, Ph.D. and Jennifer L. Bizon, Ph.D.	Department of Psychiatry Department of Neuroscience
Dietmar Siemann, Ph.D.	Department of Radiation Oncology
Colin Sumners, Ph.D.	Department of Physiology & Functional Genomics
Nihal Tumer, Ph.D.	Department of Pharmacology & Therapeutics
Stephanie Wohlgemuth, Ph.D.	Department of Animal Sciences

The Short-Term Research Training for Minority Students program is funded by a grant from the National Institutes of Health, National Heart, Lung and Blood Institute and supported by the University of Florida College of Medicine. The grant's principal investigator, Charles E. Wood, Ph.D., is the Professor and Chair for the Department of Physiology at the University of Florida.

STUDENTS	AFFILIATION
Oladele Akinsiu	University of Florida
Octavio Casanova	University of Florida
Julia Chapman	University of Cincinnati, OH
Lazaro Diaz	University of Florida
Hanns Frimpong	University of Florida
Weedley Funeus	University of Florida
Gabrielle Hall	University of Florida
Danerra Hill	Northpark University, Chicago, IL
Danielle Howard	Florida Atlantic University
Alana Jackson	Duke University, NC
Chestine Libema	University of Florida
Jasmine Nicholas	Dillard University New Orleans, LA
Steve Noutong	University of Florida
Christie Ojiaku	University of Florida
Glenn Sapp	University of Florida

SYMPOSIUM PROGRAM

BREAKFAST 8:30am

WELCOME 9:00am - 9:15am

Charles E. Wood, Ph.D., Professor and Chair for the Department of Physiology

Michelle E. Jacobs, M.D., Assistant Dean, Office of Minority Affairs and Assistant Professor, Department of Psychiatry, College of Medicine

SESSION I

SESSION CHAIR:
Dorette Ellis, Ph.D., Associate Professor, Department of Pharmacodynics

Jasmine Nicholas 9:15am – 9:30am
Project: ***"The investigation of na,k-atpase activity and the effect of riluzole on the na,k-atpase activity in mutant amyotrophic lateral sclerosis mice g93a"***
Mentor: Dorette Z. Ellis, Ph.D., Associate Professor, Department of Pharmacodynamics

Chestine Libema 9:30am – 9:45am
Project: ***"Effect of pterostilbene on drug metabolizing enzymes"***
Mentor: Reginald Frye, Pharm.D., Ph.D. Associate Professor, Department of Pharmacotherapy and Translational Research

Danielle Howard 9:45am – 10:00am
Project: ***"Cardiac-specific myosin light chain kinase plays a significant role in cardiac function"***
Mentor: Hideko Kasahara, MD, Ph.D., Associate Professor, Department of Physiology & Functional Genomics

Gabrielle Hall 10:00am – 10:15am
Project: ***"Characterization of the effects of nicotine on locomotor activity In ICR male mice."***
Mentor: Dr. Adriaan Bruijnzeel, Assistant Professor, Department of Psychiatry

Octavio Casanova 10:15am – 10:30am
Project: ***"Influence of the HMGA1 IVS5-13insC Polymorphism on New Onset Type II Diabetes Mellitus in INVEST"***
Mentor: Reginald Frye, Pharm.D., Ph.D. Associate Professor, Department of Pharmacotherapy and Translational Research

Break 10:30-11:00

SESSION II

Session chair:
Jennifer L. Bizon, Ph.D., Department Of Neuroscience

Alana Jackson 11:00am – 11:15am
Project: ***"Does the placenta express the fgf23/klotho-fgfr3 counter-regulatory mechanism for 1,25-dihydroxyvitamin d_3 synthesis?"***
Mentor: Kirk Conrad, MD, Professor, Department of Physiology & Functional Genomics

Glenn Sapp 11:15am – 11:30am
Project: ***"Adiponectin production regulation response to exercise"***
Mentor: Judy Muller-Delp, Ph.D., Associate Professor, Dept. of Physiology & Functional Genomics

Steve Noutong 11:30am – 11:45am
Project: ***"Influence of the renin angiotensin aldosterone system (raas) on the expression of gper-1, mr and 11βhsd2 in the brain."***
Mentor: Colin Sumners, Ph.D., Professor, Dept. of Physiology & Functional Genomics

Julia J. Chapman 11:45am – 12:00pm
Project: *"**Increased basal forebrain gabaergic neurons in spatially impaired aged f344 rats is not associated with a change in total neuron number"***
Mentor: Jennifer L. Bizon, Ph.D., Associate Professor, Dept. of Neuroscience

Oladele Akinsiku 12:00pm – 12:15pm
Project: ***"No sexual dimorphism in development of kidney damage in the aging fischer-344 (f344) rat kidney"***
Mentor: Chris Baylis, Ph.D., Professor, Dept. of Physiology & Functional Genomics

Lunch 12:15 – 1:15

SESSION III

SESSION CHAIR:
Charles E. Wood, Ph.D., Professor and Chair, Department Of Physiology And Functional Genomics

Hanns Frimpong 1:30pm – 1:45pm
Project: ***"The effect of traumatic brain injury on catecholamine biosynthesis in rat adrenal medulla"***
Mentor: Nihal Tumer, Ph.D., Professor, Department of Pharmacology and Therapeutics

Chrisite A. Ojiaku 1:45pm – 2:00pm
Project: ***"Nadph oxidase is necessary for optimal insulin secretion"***
Mentor: Clayton Matthews, Ph.D., Department of Pathology and Laboratory Medicine

Weedley Funeus 2:00pm – 2:15pm
Project: "***Effects of resveratrol on age-related changes in adrenomedullary catecholamine biosynthesis"***
Mentor: Nihal Tumer, Ph.D., Professor, Department of Pharmacology and Therapeutics

Danerra Hill 2:15pm – 2:30pm
Project: "***Estrogen effects on c2c12 myoblasts and saos osteosarcoma cells"***
Mentor: Stephanie Wohlgemuth, Ph.D., Lecturer, Department of Animal Sciences

Lazaro Diaz 2:30pm – 2:45pm
Project: "***Higher antioxidant capacity reduces post-irradiation oxidative stress and leads to higher mating success in anastrapha suspensa and drosophila melanogaster 1specimens"***
Mentor: Daniel A. Hahn, Ph.D., Assistant Professor, Department of Entomology and Nematology

PRESENTATION OF CERTIFICATES 2:45PM – 3:30PM

STUDENT GROUP PICTURE 3:30PM – 3:45PM

ABSTRACTS

"The Investigation of Na,K-ATPase activity and the effect of Riluzole on the Na,K-ATPase activity in mutant Amyotrophic Lateral Sclerosis mice G93A"

Jasmine Nicholas, Dorette Z. Ellis, Dept. of Pharmacodynamics

Purpose: Amyotrophic Lateral Sclerosis, ALS or Lou Gehrig's disease, is one of the most common forms of adult onset neurodegenerative disorders. It is characterized by degeneration of motor neurons in the brain and spinal cord. The death of the motor neurons leads to muscle wasting, paralysis, and death. Previous study demonstrated that there were global losses of Na,K-ATPase activity in mutant ALS mice (ALS) G93A. The purpose of this study is to investigate the role of Na,K-ATPase activity in ALS-like symptomatic L126Z-SOD1 mutant mice and wild-type mice spinal cord.

Methodology: Tissue slices from L126Z-SOD1 mutant mice and wild type spinal cord slices were incubated with agonists and antagonists in a physiological buffer. Na,K-ATPase activity was determined by assaying hydrolysis of adenosine triphosphate (ATP) in suspended permeabilized tissue slices.

Results: L126Z-SOD1 spinal cord tissue was found to have significantly lower Na,K-ATPase activity when compared to the wild-type spinal cord tissue. With the application of riluzole, the Na,K-ATPase activity in LI26Z-SOD1 spinal cord tissue decreased, when compared to its respective untreated control and in the regular wild type.

Conclusions: Decreases in Na,K-ATPase activity after the application of riluzole indicates that the drug effectively decreased Na,K-ATPase activity in spinal cord tissue in L126Z-SOD1 mutant mice. This suggests reduced usage of Na,K-ATPase by the spinal cord, thereby utilizing less energy given that in actively firing neurons. The Na,K-ATPase is responsible for 50% of energy consumed by the CNS.

"Effect of Pterostilbene on Drug Metabolizing Enzymes"

Chestine Libema, Reginald Frye, Mohamed E. Mohamed, Melonie Stanton
Department of Pharacotherapy and Translational Research

Approximately 20% of Americans consume at least one herbal supplement. Recent surveys indicate that one in four herbal supplement users also takes one or more prescription drugs, raising the potential for herb-drug interactions. Although several advances have been made in order to elucidate the impact of several herbal products on drug metabolism, there is still a need for more research, especially for herbal agents that have not been extensively studied. One such agent, pterostilbene, is a phytochemical component of berries of the Vitis and Vacciniun genera. Previous investigations have shown that this agent may confer cardio-protective, anti-diabetic, anti-inflammatory, and anti-cancer effects in humans. However, not many studies have focused on its effect on drug metabolism. The aim of this study is to determine whether pterostilbene significantly inhibits 5 drug-metabolizing enzymes of interest, CYP2C8, CYP3A4, UGT1A1, UGT1A6, and UGT1A9. The effect of pterostilbene on inhibition of each of these enzymes was studied in vitro using pooled human liver microsomes. For each inhibition assay, pterostilbene at a concentration of 1-100 μM and an enzyme-selective substrate were incubated with human liver microsomes. Samples were then analyzed using high-performance liquid chromatography tandem mass spectrometry in order to measure the amount of metabolite that was produced. The amount of metabolite produced corresponded to enzyme activity. Results revealed that pterostilbene inhibited the CYP450 enzymes and UGT1A9, but did not seem to have an effect on UGT1A1. At a concentration range of 1-100 μM, pterostilbene moderately inhibited CYP2C8, CYP3A4, and UGT 1A9 with IC50 concentrations of 26.9 μM, 207.5 μM, and 251.0 μM, respectively. Further studies remain to be done in order to discover the inhibitory effect of pterostilbene on UGT1A6.

"Cardiac-specific Myosin Light Chain Kinase Plays a Significant Role in Cardiac Function"

Danielle Howard, Hideko Kasahara, Soni Warren
Department of Physiology & Functional Genomics

Heart disease covers a vast array of diseases, such as arrhythmias, infections, and defects of the heart, and is the leading cause of death in both men and women in the United States today (causing approximately 25% of yearly deaths). Although treatments for heart disease vary depending on the specific ailment, a majority of treatments focus on reducing the work load and demands of the diseased heart, thereby increasing the efficacy of its poor function. There are very few treatments that attempt to return the heart to full function. Thus, the development of a new treatment for heart disease is a promising study.

Our lab focuses on the effect of a novel kinase on heart disease and failure. This kinase, cardiac-specific myosin light chain kinase (cMLCK), was identified in 2007. The cMLCK phosphorylates the light chains of myosin, which, in turn, regulates sarcomere assembly. The focuses of this study were directed at the effects of cMLCK in cardiac function, and thus, two types of cMLCK knockouts were generated. Germline cMLCK knockout mice on the whole displayed cardiomyopathy, characterized by a weakening and enlargement of the heart. Additionally, knockout mice displaying the inducible CRE gene were generated, allowing for control over when the cMLCK was removed. Transgenic mice were also created, with production of cMLCK upregulated 20-fold.

In this study, I compared the ejection fractions and masses of the left ventricles of 5 wildtype mice and 6 germline cMLCK knockout mice, all 3-4 month old females of BL6/129 background. The ejection fraction is defined as the percent of blood that leaves the heart after entering. It is determined by dividing the stroke volume (which is the differences in the volumes at end diastole and end systole) by the end diastolic volume. To determine the volumes, 5-6 sliced MRI images were taken of the left ventricle of each mouse, each one millimeter thick. The internal area of each slice was traced using ImageJ software at both end diastole and end systole. By subtracting the end systolic area for each slice from the end diastolic area for each slice, and then adding the results, I was able to determine the stroke volume, and thus, the ejection fraction. The mass was similarly calculated, using the conversion of 1 pixel to every 0.26458 millimeters.

From the above experiment it was determined that the ejection fraction of left ventricles in the germline knockouts was significantly lower ($p = 0.0041$) than that of the wildtype mice. However, no significant difference ($p = 0.1470$) was found in the left ventricle masses of the mice. This indicates that the cardiomyopathy of the knockout mice's hearts weakened their pumping ability.

Additionally, I worked with pressure/volume data for cMLCK knockout, wildtype, and transgenic (cMLCK upregulated) mice (all female BL6/129). The mice had all had surgery to implant a TAC band around their aortas, which restricted blood flow and simulated stress. The pressure and volume inside the heart were measured with a catheter that was inserted into the left ventricle via the carotid artery of the mice. The data obtained was then analyzed and graphed on the computer, and information about the ejection fractions and contractility were obtained.

Results from the pressure volume analysis confirmed the ejection fraction data previously obtained, showing that ejection fraction was highest in the transgenic mouse and lowest in the knockout. Additionally, it was showed that the contractility, using the wild type with TAC as a basis, increased by 15.1% in the transgenic mouse, and decreased by 39.6% in the knockout mouse. This suggests not only

that cMLCK plays a significant role in normal heart function, but also that upregulated amounts of it can preserve heart function under pathological stress conditions.

In conclusion, the results of the above study provide support for the hypothesis that cMLCK is important in heart function, as ejection fractions and contractility were lower in mice with no cMLCK. Additionally, cMLCK has promise for use as a treatment of heart disease, as mice that had overexpresion of cMLCK had higher ejection fractions and contractility.

"Characterization of the effects of nicotine on locomotor activity in ICR male mice."

Gabrielle Hall, Adriaan Bruijnzeel, Rayna Bauzo and Gene Roddick
Department of Psychiatry

Nicotine addiction is a chronic mental illness characterized by negative withdrawal symptoms, a compulsive desire for nicotine, and reintroduced craving for the drug after abstaining from smoking. Dopamine expression may be responsible for the development of nicotine dependency. It has been found that upon exposure to nicotine, the serotonergic system is activated, which appears to exhibit a large influence on dopaminergic function. It is hypothesized that the serotonin (5HT-2C) receptor can mediate dopaminergic functioning that is involved with the rewarding properties of nicotine. Past studies have revealed that 5HT-2C receptor antagonists can increase dopamine activity, causing a stimulatory effect that increases locomotor activity in mice treated with nicotine. In this study, the effect of nicotine on locomotor activity in ICR mice will be characterized. The purpose of this experiment is to investigate the effects of PAT and methyl-PAT on locomotor activity induced by nicotine use in mice. These two compounds stimulate the 5HT-2C receptor, implicating that activation of this receptor contributes to nicotine sensitization. It is expected that nicotine-treated mice will experience increased locomotor-activity compared to the saline-treated mice. This study will elucidate how activation of the 5HT-2C receptor may produce nicotine sensitization, and help to understand methods for minimizing the rewarding effect of nicotine. This could potentially lead to new therapies for treating nicotine use in humans.

"Influence of the HMGA1 IVS5-13insC Polymorphism on New Onset Type II Diabetes Mellitus in INVEST"

Octavio Casanova, Reginald Frye, Jason Karnes, Taimour Langaee, Julie Johnson, and Rhonda Cooper-DeHoff.
Department of Pharmacotherapy and Translational Research.

Type 2 Diabetes Mellitus (T2DM) is a complex disease affecting over 250 million people worldwide. It involves many organs and organ systems and has genetic as well as environmental components. The HMGA1 protein is a structural protein that regulates transcription of many genes, one of which is the insulin receptor gene. The insulin receptor plays a central role in glucose homeostasis and, thus, development of T2DM. Studying the HMGA1 gene may reveal a link between HMGA1 and T2DM. This project samples 1680 individuals in a 3:1 case control analysis, where the cases are those individuals who have recently developed T2DM while participating in the INVEST study. The individuals are genotyped for a particular single nucleotide polymorphism (SNP) of HMGA1; a C insertion 13 bases upstream of exon 6. Those with the polymorphism (which occurs only on one copy of the chromosome and is thus a heterozygous mutation) and those individuals with the wild-type allele are analyzed for a possible association between T2DM and the HMGA1 SNP. Statistical analysis, including linear regression as well as chi-squared analysis, currently show no significant association between T2DM and the HMGA1 polymorphism studied, though the data is largely preliminary. However, there is some significant difference in polymorphism prevalence by race that is likely worth exploring further. An increase in the sample size, as well as further grouping of cohorts by race using more stringent parameters, may produce more significant results. In addition, two homozygotes have been identified, and this polymorphism was previously thought to be lethal. Further study of this group may reveal some new associations worth investigating.

"Does the Placenta Express the FGF23/Klotho-FGFR3 Counter-regulatory Mechanism for 1,25-dihydroxyvitamin D_3 Synthesis?"

A. Jackson, E. Sumners, and K.P. Conrad
Departments of Physiology and Functional Genomics, OBGYN

During normal pregnancy, elevated maternal plasma concentrations of 1,25 dihydroxyvitamin D_3 ($1,25OHD_3$) increase intestinal calcium absorption leading to "physiologic absorptive hypercalciuria". Like many maternal adaptations to pregnancy, this response begins in the first trimester, thus anticipating fetal calcium demands for skeletal formation later in gestation. In addition to the kidney, the human placenta synthesizes $1,25OHD_3$ and expresses the vitamin D receptor (VDR). Thus, the elevated levels of circulating $1,25OHD_3$ during gestation may be placental in origin. In contrast, preeclampsia, which occurs in ~ 5% of pregnancies and is characterized by new onset hypertension and proteinuria beginning in the late second or third trimester, is characterized by decreased placental production and circulating levels of $1,25OHD_3$ leading to relative hypocalciuria.

The etiology of preeclampsia remains largely unknown, but has been associated with inadequate trophoblast invasion of the uterus, and consequently, "shallow" placentation. One hypothesis is that deficiency of placental $1,25OHD_3$ contributes to incomplete decidualization and immune suppression leading to inadequate trophoblast invasion and placentation. For this reason, it is important to look at the molecules that regulate the production of $1,25OHD_3$. Fibroblast growth factor (FGF) 23 is a circulating hormone produced in the bone that acts on the kidney to inhibit CYP27B1 and stimulate CYP24R1, thus suppressing production of $1,25OHD_3$, thereby regulating calcium homeostasis by the gut. FGF23 activates a Klotho-FGFR3 heterodimer in the kidney.

We hypothesized that the FGF23/Klotho-FGFR3 counter-regulatory system also exists in the normal human placenta, and is overexpressed in the the preeclamptic placenta. To begin investigating this overarching hypothesis, we extracted RNA from HTR-8/SVneo first trimester extravillous trophoblast cells, synthesized cDNA, and then performed RT-PCR. We utilized specific primers to amplify Klotho (the soluble form of which has been detected in umbilical cord blood), Klotho, FGF23, and FGFR3, as well as the CYP enzymes involved in $1,25OHD_3$ synthesis and metabolism, and VDR.

Results are pending, but if the FGF23/Klotho-FGFR3 counter-regulatory system is expressed in trophoblast cells, then in future investigations we will (1) knock-down this system and determine whether CYP27B1 and CYP24R1 are up- and downregulated, respectively, and (2) test whether there are quantitative differences between normal pregnant and preeclamptic placentas, i.e., exaggerated expression in preeclamptic placentas which could explain the reduced production of $1,25OHD_3$.

"Adiponectin Production Regulation Response to Exercise"

Glenn Sapp, Judy Muller-Delp
Department of Physiology and Functional Genomics

Background and significance: Adiponectin, an adipokine, interacts with various functional systems to regulate vascular homeostasis. Synthesis of the protein occurs in adipocytes, and it circulates in three oligomeric forms. Adiponectin activates receptors located in the brain, liver, reproductive tract, and the vasculature. Increased levels of adiponectin have been reported to reduce platelet aggregation, deactivate reactive oxygen species(ROS), and increase endothelium-derived nitric oxide production. Clinically, reduced circulating adiponectin is associated with atherosclerosis, diabetes, and cardiovascular disease. Interestingly, numerous reports indicate that as adipose tissue mass decreases, circulating adiponectin increases, suggesting that the metabolic and cardiovascular benefits associated with increased circulating adiponectin may not be attributed to adipocyte-derived adiponectin in ameliorating . The purpose of this study was to determine whether the vasculature is a source of adiponectin synthesis.

Hypothesis: The purpose of this study was to test the following hypotheses: 1) Adiponectin is produced locally in vascular tissue. 2) The vascular endothelium of resistance arteries synthesizes adiponectin. 3) Aerobic exercise training increases vascular synthesis of adiponectin

Methods: Resistance arteries were isolated from cardiac and skeletal muscle of Fischer 344 rats, and real-time RT-PCR was performed to assess adiponectin mRNA. To specifically identify adiponectin mRNA in the endothelium and/or vascular smooth muscle of resistance arteries, RT-PCR was performed in intact resistance arteries and in arteries denuded of endothelium. In addition, adiponectin mRNA was assessed in cultured endothelial cells exposed to shear stress or maintained under static conditions. Finally, adiponectin mRNA expression was determined in skeletal muscle resistance arteries from rats that either performed aerobic exercise training or remained sedimentary for 10 weeks.

Results: Adiponectin mRNA was detected in both intact and endothelium-denuded resistance arteries, indicating that both the vascular endothelium and the vascular smooth muscle are sources of adiponectin synthesis. In cultured endothelial cells, exposure to shear stress increased adiponectin mRNA expression. Adiponectin mRNA expression was significantly increased in skeletal muscle resistance arteries from exerecise-trained rats as compared to arteries from sedentary rats.

Conclusions: These data indicate that the resistance vasculature is a source of locally produced adiponectin in cardiac and skeletal muscle. Adiponectin is expressed in both the endothelium and vascular smooth muscle of resistance arteries, and this expression is modulated acutely by exposure to shear stress and chronically by performance of regular aerobic exercise.

"Influence of the Renin Angiotensin Aldosterone system (RAAS) on the expression of GPER-1, MR and 11βHSD2 in the brain"

Steve Noutong, Peng Shi, Elaine Sumners and Colin Sumners*
Department of Physiology & Functional Genomics

Background and Hypothesis The brain plays an important role in the control of blood pressure. The paraventricular nucleus (PVN) of the hypothalamus plays a key role in the brain control of blood pressure, via stimulatory effects on both the sympathetic outflow and the hypothalamus-pituitary axis. It is clear that angiotensin II (Ang II) can act at its neuronal AT1 receptor in the PVN to increase sympathetic outflow and raise blood pressure. More recently, our lab has shown that this action of Ang II in the PVN is enhanced in hypertension, and that this enhancement involves recruitment and activation of immune/inflammatory cells such as microglia at this site. It is established that mineralocorticoid hormones have a close association with the renin angiotensin system, and well known that these steroids have a role in the inflammatory processes that contribute to cardiac fibrosis. Based on this we speculated that mineralocorticoids and their receptors might play a role in the inflammatory mechanisms in the PVN that contribute to Ang II induced hypertension. Specifically, we hypothesized that increased activity of the RAAS in the brain might increase the expression of the traditional mineralocorticoid receptors (MR), and also the expression of Gper30 (GPER-1), which has been proposed as a mediator of rapid (non-genomic actions) of the mineralocorticoid aldosterone.

Aims Determine the relative expression of GPER-1 and MR in the PVN of sRA mice. These double transgenic mice, which have overexpression of human renin under the control of a neuron-specific synapsin promoter (**sR**) and human angiotensinogen (**A**), exhibit *hyperactivity* of the renin-angiotensin system in specific brain areas (AV3V and hypothalamus), but depressed RAAS activity in the periphery. These sRA mice develop chronic high blood pressure associated with increased sympathetic nerve activity. Additionally, to determine the relative expression GPER in the PVN of mice made hypertensive via subcutaneous administration of the mineralocorticoid deoxycorticosterone acetate (DOCA; 50mg, 21days) plus 0.15 mM NaCl to drink.

Methods Following extraction and purification of RNA from the mice' PVNs, cDNA was obtained by reverse transcription using a high capacity cDNA archive kit. Subsequently, Real Time PCR was used to detect and quantify the various genes of interest. The average threshold cycle (Ct) for each gene was obtained in respective subjects and subtracted from the corresponding Ct value of the 18s Ribosomal normalizer to acquire the ΔCt values. Using SigmaStat, T-tests were then run between different groups to assess their degree of difference.

Results In the wild type (n=6) and transgenic male mice (n=5), the average ΔCt values were respectively found to be 8.780±0.315 and 8.945 ±0.515 (P=0.783) for GPER-1, 6.987±0.330 and 7.246 ±0.566 (P=0.690) for MR and 14.430 ±0.448 and 14.371±0.329 (P=0.921) for the cortisol-inactivating enzyme 11βHSD2. The average ΔCt values in wild type (n=5) and transgenic female mice (n=5) were respectively found to be 9.906 ±0.291 and 9.565±0.701 (P=0.665) for GPER-1, 12.140±0.416 and 11.489 ±0.562 (P=0.379) for MR and 15.038 ±0.296 and 14.947 ±0.508 (P=0.881) for 11βHSD2. The average ΔCt value of GPER-1 in the DOCA/Salt treated (n=4) and non-treated (n=3) mice was respectively 12.662 ±0.0417 and 12.064 ±0.353 (P=0.213).

Conclusion: The results suggest that there is no significant difference in GPER-1, MR and 11βHSD2 expression in the PVN of double transgenic (sRA) mice. Additionally, we reported no significant change in the expression of GPER-1 receptor gene in DOCA-salt treated mice. However, trends in the data and high standard error bars might imply that more sizable samples would be essential to reach a definitive conclusion.

"Increased basal forebrain GABAergic neurons in spatially impaired aged F344 rats is not associated with a change in total neuron number"

J. J. Chapman, C. Bañuelos, C. L. LaSarge, J. L. Bizon
Department of Neuroscience

The basal forebrain is an integral part of a complex circuitry that controls cognitive functions such as emotions, learning and memory in the mammalian brain. Dysfunction of basal forebrain systems has been implicated in memory decline in aging although that vast majority of such studies have focused on the basal forebrain neurons of the cholinergic phenotype. Co-distributed GABAergic neurons in the rostral basal forebrain are an integral part of this system and these neurons are equally well positioned to impact cognitive processes. In the current study, distinct populations of basal forebrain neurons were quantified in behaviorally characterized young and aged F344 rats. Using the water maze, approximately half of the aged rats demonstrated spatial learning performance on par with young rats (aged cognitively-unimpaired), whereas others fell outside the range of young, demonstrating cognitive impairment (aged cognitively-impaired). We have previously shown that while ChAT+ neuron number is modestly reduced as a function of age (but not cognitive status), there is a selective and marked *increase* in GABAergic neurons (identified with GAD67 immunofluorescence) in the rostral basal forebrain of aged cognitively-impaired rats compared to young and aged cognitively-unimpaired rats. These data demonstrate a strong relationship between changes in GABAergic basal forebrain neurons and impaired spatial learning at advanced ages in F344 rats and suggest that age-related alterations in GABAergic neurotransmitter systems may contribute to mnemonic dysfunction in aging. In order to determine whether the increase in GABAergic basal forebrain neuron number in aged cognitively-impaired rats reflected a change in overall neuron number in this region, the current study used stereology to quantify the total number of neurons (identified with NeuN immunofluorescence)in these same basal forebrain subfields. Neuronal estimates of NeuN+ neurons indicate that there is no change in overall neuron number among age and cognitive groups. These data suggest that the increased GABAergic cell number observed in aged cognitively-impaired rats is not due to an addition of new neurons, but may instead reflect an upregulation of GABA synthesis in existing neurons that is associated with memory impairment.

"No sexual dimorphism in development of kidney damage in the aging Fischer-344 (F344) rat kidney"

Oladele Akinsiku[1], Jennifer Sasser[1], Natasha Moningka[1], Katie Jerzewski[1], Amanda Jo LeBlanc[2], Lori Kang[2], Amy Sindler[2], Judy Muller-Delp[1,2], Chris Baylis[1]
Physiology and Functional Genomics

The aging kidney exhibits a fall in glomerular filtration rate due to renal vasoconstriction and structural damage. Females are usually protected against kidney injury as compared to males, in part because of cardiorenal protective effects of estrogens. Systemic and renal nitric oxide (NO) deficiency also contributes to age-dependent kidney damage in rats. In this study, we compared intact, ovariectomized (OVX) and ovariectomized + estrogen-replaced (OVE, 2x 0.05 mg 17β-estradiol 60-day slow-release pellets, Innovative Research) young (Y; 6mo) and old (O; 24m) female rats with Y and O intact males by measuring the kidney injury and protein abundance of endothelial NO synthase (eNOS), neuronal NO synthase alpha (nNOS α), and neuronal NO synthase beta (nNOS β) in both the kidney cortex and medulla. Unexpectedly, there was no difference in age-dependent kidney damage between Y or O intact M and F F344 (Table). Furthermore, neither Ovx nor Ovx + estrogen replacement had an impact on the kidney injury. YM had *greater* KC eNOS abundance than YF, and this difference persisted in OM vs OF. In contrast, there was a decline in eNOS in the renal medulla with age in M but not F. . While kidney nNOS alpha expression was not different in M or F with age, there was a significant increase in nNOS beta abundance in OM. In conclusion, the kidney damage expressed in the aging F344 is fairly mild, develops similarly in M and F and is not related to renal cortical loss of eNOS or nNOS protein. This is in contrast to the aging SD M rat (Erdely et al, 2003) where kidney damage is exacerbated and eNOS and nNOSα is lost compared to the OF. The lack of effect of Ovx or estrogen replacement on OF suggests that in F344 ovarian hormones do not influence these aspects of kidney aging.

*p<0.05 vs respective Y, + p<0.05 vs intact F of same age.

	% damaged glomeruli	Glomerular Sclerosis Index (GSI, 1-4 scale)	Casts (#)	eNOS cortex	eNOS medulla	nNOS alpha cortex	nNOS alpha medulla	nNOS beta cortex	nNOS beta medulla
YM	2.1±0.6	0.03±0.01	0.3±0.2	1.9±0.4 +	0.8±0.1	0.63±0.1	0.8±0.1	0.8±0.1	1.0±0.2
OM	12.7±2.3 *	0.27±0.06 *	10.0±2.9 *	2.6±0.5 +	0.4±0.1 *	1.6±0.3	0.5±0.1	5.9±1.2*	3.0±0.6 *
YF	2.0±0.2	0.02±0.01	0.0±0.0	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1
OF	17.0±2.0 *	0.38±0.05 *	14.0±4.9 *	0.9±0.2	0.7±0.1	0.9±0.2	1.1±0.1	1.9±0.4	2.0±0.3
YF-OVX	2.7±0.3	0.04±0.01	0.1±0.1	1.5±0.2	1.2±0.2	0.9±0.1	1.5±0.2	0.8±0.1	1.0±0.2
OF-OVX	17.4±5.6 *	0.41±0.16 *	12.7±5.6	2.4±0.3 +	0.9±0.1	1.6±0.3	1.5±0.2	1.4±0.3	1.8±0.3
YF-OVE	1.9±0.4	0.02±0.01	0.8±0.8	2.7±0.4 +	1.1±0.1	1.9±0.3	1.7±0.2 +	1.1±0.3	2.0±0.2
OF-OVE	10.2±0.7	0.20±0.03	11.8±3.3	3.4±0.4 +	0.7±0.1	1.8±0.2	1.1±0.1 *	1.9±0.5	2.4±0.4

"The Effect of Traumatic Brain Injury on Catecholamine Biosynthesis In Rat Adrenal Medulla"

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There is a high incidence of traumatic brain injury (TBI) among soldiers returning from war-time deployment. The severity of the injury often produces major health problems for those individuals suffering from TBI. It has been shown that TBI disrupts noradrenergic pathways leading to autonomic dysfunction but the nature of this disruption is unknown. Therefore, we assessed selective biochemical markers for autonomic function in adult female Sprague Dawley rats following TBI. Closed head TBI was produced using the Marmarou protocol (400 g/1.25 m weight drop). Eight weeks following injury, animals were sacrificed and the adrenal glands were removed. Prior to analysis the glands were decapsulated so that the medullae could be separated from the cortex. Western blot and semi-quantitative RT-PCR were used for the assessment of protein and mRNA levels of catecholaminergic biosynthetic enzymes; tyrosine hydroxylase (TH) and dopamine-β hydroxylase (DβH), along with Neuropeptide Y (NPY), Angiotensin-II Type 1 (AT1) and Angiotensin-II type 2 (AT2) receptors. TH mRNA and DβH protein expression increased by 55% ($P<0.05$) respectively following TBI. While there were no significant differences in AT1 and AT2 receptor expression following injury, NPY expression was significantly elevated by 60% ($P<0.05$) in TBI rats compared with age-matched controls. NPY is synthesized and co-released with catecholamines in the adrenal medulla and NPY expression correlates with catecholamine biosynthesis. Taken together, the increased TH, DβH and NPY expression in the rat adrenal medulla suggests that closed head TBI results in increased sympathoexcitation. Increased sympathoexcitation may be one of the important factors contributing to autonomic dysfunction following TBI.

"NADPH Oxidase is Necessary for Optimal Insulin Secretion"

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Reactive oxygen species (ROS) are essential for many processes from cardiovascular function to the elimination of pathogens by the immune system. Excessive ROS production is a major factor in the progression of chronic diseases, including diabetes mellitus. While these molecules can damage tissues, cells can lose full functionality when ROS production is blocked. Recent reports using pharmacological inhibitors of NADPH Oxidase (NOX), a superoxide-generating enzyme that is a major source of cellular ROS, have suggested a role for NOX in glucose stimulated insulin secretion by pancreatic beta cells. However, the inhibitors used in previous studies lack specificity and may block other pathways important for insulin secretion. To directly examine the role of superoxide produced by NOX in insulin secretion, we used mice with a genetic ablation in *Ncf1*, the regulatory subunit of NOX. These *Ncf1*^{m1J} mutant mice have abolished NOX activity. We hypothesize that these mutant mice will have blunted insulin secretion due to reduced ROS production.

For these studies we used wild type or *Ncf1*^{m1J} mutant mice. Mice were subjected to glucose tolerance tests for assessment of insulin secretion, beta cell insulin secretory function in response to secretagogues, and content of both immature and mature insulin were measured in isolated islets. RNA was extracted from isolated islets using Trizol, and cDNA was synthesized. cDNA was used as a template in Real-Time PCR to determine the message levels for insulin, an insulin specific transcription factor [Pancreatic and duodenal homeobox 1 (Pdx-1)], and a marker of endoplasmic stress [C/EBP homologous protein (CHOP)].

First phase glucose-stimulated insulin secretion was assessed in the *Ncf1*^{m1J} and *Ncf1* intact mice, *in vivo*, where a 60% reduction in first phase insulin secretion in the *Ncf1*^{m1J} mice was measured. *In vitro* experiments confirmed the defect. Isolated pancreatic islets were exposed to either high glucose (16.7mM) or potassium (KCl, 20mM). While no difference in insulin secretion was observed in response to KCl, *Ncf1*^{m1J}-mutant islets secreted significantly less insulin in response to glucose challenge. To test if NOX played an acute role in insulin secretion we tested the effect of a superoxide scavenger (FCB007) on glucose stimulated insulin secretion. FCB007 had no effect on glucose stimulated insulin secretion, suggesting that superoxide is not essential for the acute effect of glucose on insulin secretion. To further define the defect, we measured total insulin and proinsulin content in isolated islets by ELISA. Insulin content of the *Ncf1*^{m1J}-mutants was significantly higher than the WT, although the proinsulin content was not statistically different. RNA transcript levels for insulin and Pdx-1 were comparable in islets from both strains, however CHOP was increased three-fold in Ncf1^{m1J} islets.

Due to the significant reduction in insulin secretion we conclude that NOX [and possibly superoxide] is necessary for normal insulin secretion by beta cells. As there was no reduction in insulin or proinsulin content in *Ncf1*^{m1J} mutant islets and levels of insulin and Pdx-1 mRNA between the two strains were similar, we conclude that the impact of NOX in the beta cell is upstream of the insulin transcription and translation. However, the similarity of insulin secretion in response to KCl would suggest the difference is prior to the final stage of insulin secretion. Based on the data where CHOP is elevated we suggest that NOX plays a role in the post-translational modification of insulin and the elevation of ER stress due to the absence of NOX negatively impact insulin secretion both *in vitro* and *in vivo*.

"Effects of Resveratrol on Age-Related Changes in Adrenomedullary Catecholamine Biosynthesis"

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Resveratrol is a natural phenol found in significant amounts in red wine. Resveratrol has been shown to possess anti-inflammatory, anti-cancer, and anti-aging effects. To better understand its role in the anti-aging process, we examined if resveratrol reverses the age-related increase in catecholamine biosynthesis in the adrenal medulla. Male Fischer 344 x Brown Norway rats were separated into four groups; young control, old control, young resveratrol and old resveratrol. Resveratrol (15mg/kg) was put in the drinking water of the young and old resveratrol groups for 14 days. Tyrosine hydroxylase (TH), dopamine-beta hydroxylase (DβH) and neuropeptide Y (NPY) were measured in the adrenal medulla. Expression of TH and DβH were significantly higher in the old rats ($P<0.05$). However, reseveratrol treatment significantly lowered TH and DβH in the old animals ($P<0.05$). NPY was also higher in the old rats when compared with the young animals, and reseveratrol treatment significantly reversed the age-related up-regulation of NPY in the old rats ($P<0.05$). This data suggests that resveratrol treatment may be beneficial in reversing the age-related increase in adrenomedullary catecholamine biosynthesis.

“Estrogen effects on C2C12 myoblasts and SAOS osteosarcoma cells”

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Discoveries have shown that hormones such as estrogen have a role in the onset and development of adolescence idiopathic scoliosis (AIS). Studying the role of estrogen is relevant to understand the interaction with potential factors that influence the development and progression of this disease. Overall we know that estrogen impact bone remodeling, growth, as well as bone acquisition all of which are abnormal in AIS. It is widely accepted that progression of scoliosis manifests during skeletal bone maturation, pubertal sprouts that normally occur during teenage years and that estrogen are important during development. Investigating the effects of estrogen on both skeletal muscle and bone cells is necessary to better understand how estrogen contributes to bone and muscle growth and remodeling especially during pubertal years. According to literally research it was shown that over expression of estrogen-induced genes (EIG121) regulates autophagy and promotes cell survival under starvation stressors and exposure to cytotoxic agents. Autophagy (self-eating) responds to a stress adaptation and avoids cell death. As a first step we aimed to determine the effect of estrogen at physiological concentration of $10^{-9}M\ and\ 10^{-8}M$ of 17β estradiol) on autophagy and mitochondrial function in myoblasts (C2C12) and osteosarcoma (SAOS) cells. A second goal was to investigate whether estrogen has a protective effect when cells are exposed to a stressor, such as the oxidant H2O2, and whether this potential effect is mediated through autophagy.

We used C2C12 myoblasts, and SAOS osteosarcoma cells to test the effect of estrogen on autophagy and mitochondrial respiration. We hypothesized that estrogen would stimulate autophagy. As a second hypothesis we proposed that estrogen provides stress resistance when cells were exposed to a stressor such as H2O2, maybe through increased autophagy. However, we had no prediction about its effect on mitochondrial function. As a first step, we induced autophagy in the absence of estrogen using amino acid free medium. We monitored autophagy by imaging fluorescent signals from 1) monodansylcadaverine (MDC), added to the C2C12 growth medium at the beginning of a starvation experiment, or 2) imaging the GFP-LC3 signal in SAOS cells. We furthermore tested whether we could use the change in overall GFP signal to detect stimulated autophagy in a (Microplate) spectrophotometer. We also determined cell survival using a spectrophotometric resazurin assay. Mitochondrial respiration was measured in permeabilized C2C12 and SAOS cells using a high resolution respirometer.

Higher antioxidant capacity reduces post-irradiation oxidative stress and leads to higher mating success in *Anastrapha suspensa* and *Drosophila melanogaster specimens.*

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Invasive insects are a constant threat in today's global economy. These insects arrive through our ports and can become established, damaging crop production and reducing export options, thus reducing profitability. Without the constant pressure of their natural predators, these animals can reproduce quickly and uncontrollably. The cost of dealing with invasive insect species like fruit flies, for example, is over 4 million dollars each year in Florida alone, and is substantially more worldwide. The sterile insect technique (SIT) is a non-pesticidal area-wide control method that can be effective in preventing the establishment of invasive fruit fly pests. This technique consists of sterilizing large numbers of flies using gamma radiation and then releasing them into the target areas to mate with wild flies, thus causing them to produce infertile eggs and decreasing populations. While gamma irradiation efficiently induces sterility, insects also endure extensive somatic cellular damage that significantly decreases their flight and mating performance. Gamma radiation causes an increase in reactive oxygen species (ROS) that attack proteins, lipid membranes, DNA, RNA, and can trigger a chain reaction that results in apoptosis. One of the cell's defenses against ROS is antioxidant enzymes (AOEs). AOEs break down ROS and prevent protein, lipid, and DNA damage. Gamma irradiation leads to oxidative stress; an imbalance where ROS out number normal AOE levels. In our experiments, we show that exposing the Caribbean fruit fly, Anastrepha *suspensa,* to anoxia both prior and during gamma irradiation results in increased AOE activity. Our data demonstrates that male flies irradiated in anoxia have greater mating success, greater flight ability, and are longer lived than regular non-irradiated male flies. We set out to test the hypothesis that insects producing greater quantities of antioxidants will have less post irradiation oxidative stress. To increase AOEs without exposing flies to anoxia, we used the Gal4, UAS binary system to over express two antioxidant enzymes, SOD1 and SOD2, in *Drosophila melanogaster.* We are currently testing the parental Gal4 and UAS lines as well as the crosses for AOE activity. If we find out that these crosses have enhanced AOE activity, we can use then to test the hypothesis that over expressing AOEs can lead to better post irradiation sterile male performance. If our data indicates that AOE over expression enhances post-irradiation male performance in *Drosophila,* we will take the AOE over expression approach a step further and make transgenic lines of *A. suspensa* to evaluate the efficacy of transgenic over expression of AOEs for improving the SIT.