NIH SHORT-TERM RESEARCH TRAINING FOR MINORITY STUDENTS

SYMPOSIUM PROGRAM & ABSTRACTS
COMMUNICORE BUILDING
ROOM CG-56

UF | UNIVERSITY of FLORIDA

College of Medicine

AUGUST 10, 2012
8:00 A.M.

University of Florida College of Medicine
Office of Diversity and Health Equity

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
Michael Clare-Salzler, M.D.	Department of Pathology
Kirk Conrad, MD	Department of Physiology & Functional Genomics
Judy Delp, Ph.D.	Department of Physiology & Functional Genomics
David Fitzgerald, M.D.	Department of Neurology
Reginald Frye, Ph.D.	Department of Pharmacotherapy and Translational Research
Maria Grant, Ph.D.	Department of Pharmacology and Therapeutics
Linda Hayward, Ph.D.	Department of Physiological Sciences,
Nancy Hardt, M.D.	Department of Pathology and ObGyn
Abraham Hartzema, Ph.D.	Department of Pharmaceutical Outcomes and Policy
Julie Johnson, PharmD.	Department of Pharmacotherapy and Translational Research
Alexandra Lucas, M.D.	Department of Medicine, Division of Cardiovascular Medicine
Debbie Scheuer, Ph.D.	Department of Physiology & Functional Genomics
Elaine M. Sumners, Ph.D.	Department of Pharmacodynamics
Charles E. Wood, Ph.D.	Department of Physiology & Functional Genomics

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
Tyler Alexander	Morehouse College
Bertha Campo	University of Florida
Sofia Garcia	University of Florida
Ashlyn Goodwin	Tuskegee University
Beulah Joseph	University of South Florida
Lianette Lozada	University of Puerto Rico
Osinakachukwu Mbata	University of Georgia
Lorraine Nieves	University of Puerto Rico
Steve Noutong	University of Florida
Kimberley Panther	University of Connecticut
Maria Rojas	University of Florida
Glenn Sapp	University of Florida
Jared Smith	University of Florida
Daniel Zambrano	University of Florida

SYMPOSIUM PROGRAM

BREAKFAST AND WELCOME 8:00am

Peter Sayeski, Ph.D., Professor and Chair for the Department of Physiology

Michelle E. Jacobs, M.D., Assistant Dean, Office of Diversity and Health Equity
Professor, Department of Psychiatry, College of Medicine

SESSION I

SESSION CHAIR: CHRIS BAYLIS, Ph.D.

Bertha Campo 8:30am – 8:40am
Project: ***"Developing Novel Therapeutics for Preeclampsia"***
Mentor: Kirk P. Conrad, M.D., Professor, Physiology and Functional Genomics

Kimberley Panther 8:45am – 8:55am
Project: ***"Juvenile Offenders in Florida: A Study of the Relationship between Adverse Childhood Experiences and Truancy."***
Mentor: Nancy Hardt, M.D., Department of Pathology and ObGyn

Sofia Garcia 9:00am – 9:10am
Project: ***"Association of CYP2D6 gene copy number with Metoprolol response in the Pharmacogenomic Evaluation of Antihypertensive Responses 2 (PEAR2) Study"***
Mentor: Julie Johnson, Ph.D., Department of Pharmacotherapy and Translational Research

Ashlyn Goodwin 9:15am – 9:25am
Project: ***"Occlusion of the BC Artery May Lead To Apoptosis by Caspase 3".***
Mentor: Charles E. Wood, Ph.D., Department of Physiology and Functional Genomics

Beulah Joseph 9:30am – 9:40am
Project: ***Effects of Ifnar1 Antibody in Reducing the Type I Interferon Signaling Pathway***
Mentor: Michael Clare-Salzler, M.D., Department of Pathology

Lianette Lozada 9:45am – 9:55am
Project: ***The role of corticotrophin releasing hormone in the amygdala on the cardiovascular stress response in the shrs***
Mentor: Linda Hayward, Ph.D., Department of Physiological Sciences, Veterinary Medicine

Lorraine Nieves 10:00am – 10:10am

Project: ***Effect of Macrophage Migration Inhibitor Factor (MIF) overexpression on neuronal activation during stress responses.***

Mentor: Debbie Scheuer, Ph.D., Department of Physiology and Functional Genomics

Osinakachukwu Mbata 10:15am – 10:25am

Project: ***Programming human hematopoietic stem cells for generation of brown adipose tissue***

Mentor: Maria Grant, Ph.D., Professor, Pharmacology and Therapeutics

BREAK 10:25am– 10:45am

SESSION II

SESSION CHAIR: JUDY DELP, Ph.D.

Steve Noutong 10:45am – 10:55am

Project: ***Significance of Type III Virchow-Robin spaces in the brainstem in mild Traumatic Brain Injury (TBI)***

Mentor: David Fitzgerald, M.D., Department of Neurology

Tyler Alexander 11:00am – 11:10am

Project: ***"Renal Tubule Protein Profile in Chronically Vasodilated Rats"***

Mentor: Chris Baylis, Ph.D., Professor, Physiology and Functional Genomics

Maria Rojas 11:15am – 11:25am

Project: ***"Effects of stretching exercise on endothelium-dependent vasodilation and endothelial nitric oxide synthase expression in rat skeletal muscle arterioles"***

Mentor: Judy Delp, Ph.D., Department of Physiology and Functional Genomics

Glenn Sapp 11:30am – 11:40am

Project: ***ACE2 activation ameliorates endothelial function in the right ventrticles of pulmonary hypertensive rats***

Mentor: Judy Delp, Ph.D., Department of Physiology and Functional Genomics

Jared Smith 11:45am – 11:55am

Project: ***The Effects of Viral Proteins Serp-1 and M-T7 on Gene Expression in Mononuclear Blood Cells Isolated from Patients with Chronic Transplant Vasculopathy***

Mentor: Alexandra Lucas, M.D., Department of Medicine, Div. of Cardiovascular Medicine

Daniel Zambrano 12:00pm – 12:10pm

Project*:* ***A pharmacoepidemiological approach to the use of The bradford hill criteria of biological plausibility in Pharmacotherapy, and its application in establishing a Causal relationship between drug administration and Adverse drug events***

Mentor: Abraham Hartzema, Ph.D., Department of Pharmaceutical Outcomes and Policy

Lunch **12:30 – 1:15**

Physiology Conference Room, M559

PRESENTATION OF THE CERTIFICATES **1:15PM – 1:30PM**

PHOTOGRAPHS **1:30PM**

ABSTRACTS

Developing Novel Therapeutics for Preeclampsia

Bertha Campo, and Kirk P Conrad MD
Department of Physiology and Functional Genomics, University of Florida

Preeclampsia (PE), the major cardiovascular disease of pregnancy, causes significant maternal, fetal and neonatal, morbidity and mortality. Moreover, women and their children who suffer PE are at increased risk for developing cardiovascular disease later in life. Unfortunately, there are still no prophylactic, specific therapeutic or curative treatments for PE other than delivery. Inadequate uterine trophoblast cell invasion and spiral arterial remodeling leading to poor placentation, as well as accelerated trophoblast apoptosis are widely believed to play a role in disease pathogenesis.

Relaxin (RLX) emanates from the corpus luteum during pregnancy contributing to the marked vasodilation of the maternal circulation. RLX vasodilatory action is in part mediated through activation of PI3K/Akt and increased nitric oxide (NO) production in endothelial cells. PI3K/Akt is also a well-known anti-apoptotic pathway. In the same vein, human trophoblast cells express erythropoietin (EPO)/ EPO receptor mRNA, protein and activity, as well as b-common receptor mRNA. EPO has been shown to protect cells against apoptosis caused by ischemia-reperfusion injury through activating the b-CR/EPO-R heterodimer and the PI3K/Akt pathway. The peptide mimetic, Brines' helix B surface peptide (HBSP), acts as a specific EPO-βCR agonist without stimulating the classic EPO-R homodimer and erythropoiesis. In this study we hypothesize that RLX, HBSP or the combination will attenuate apoptosis in HTR-8/SVneo first trimester extravillous trophoblast cells subjected to hypoxia-reoxygenation injury; an adverse condition that models the ischemia-reperfusion injury of placental trophoblasts in preeclampsia.

HTR-8/SVneo cells were cultured under standard conditions, or in serum-free media and 1% oxygen. Pre-treatment with rhRLX (300ng/mL) was instigated 8 hrs prior to serum-starvation and again after changing to serum-free media. HBSP (25ng/mL) was only added after changing to serum-free media. All cells were serum-starved for 17 hrs before being placed in 1% oxygen for an additional 8 hrs, After the 8 hr hypoxic period, the appropriate media and treatments were replenished. Fixing and staining for apoptosis and nuclei using TUNEL and Hoechst dye, respectively, was performed 18 hrs later. Percent apoptosis was calculated as a ratio of TUNEL-stained nuclei to total Hoechst-stained nuclei. The preliminary data show that cells cultured under standard conditions (n=6 wells) yielded 1.06 ± 0.12% (SEM) and serum starvation with hypoxia (n=5) 5.78 ± 1.02% cell apoptosis. The rhRLX treatment group (n=5) reduced apoptosis to 3.61 ± 0.28%. The combined rhRLX+HBSP (n=6) treatment group slightly decreased apoptosis to 5.14 ± 0.69%, and the HBSP-alone treated cells (n=3) were similar to the non-treated cells, 5.66 ± 1.35% apoptosis.

Summary: On balance, the results so far suggest that RLX attenuates trophoblast apoptosis in response to hypoxia-reoxygenation *in vitro*. However, additional experiments are clearly needed, in order to increase the N number and substantiate this finding. Moreover, the methodological approach used in this study proved to have several pitfalls, and thus, flow cytometry assay of cell apoptosis is also planned to corroborate the present results. We previously provided evidence that RLX stimulates trophoblast invasion *in vitro*. In view of RLX's vasodilatory attributes, as well as the emerging evidence that it may stimulate invasion and reduce apoptosis of trophoblasts, the hormone or small molecule mimetic may be a promising therapeutic in PE.

"Juvenile Offenders in Florida: A Study of the Relationship between Adverse Childhood Experiences and Truancy"

Kimberley Panther, Nancy Hardt, M.D.
Departments of Pathology and Ob-Gyn

Background: Child maltreatment is the willful neglect and/or abuse of children – which may be sexual, physical, emotional, or supervisory in nature. Adverse childhood experiences (ACEs) are the result of child maltreatment. Historically, studies of adverse childhood experiences have been conducted on adults whose recollections serve as the basis for assessment of ACEs. ACEs have been primarily linked to studies of morbidity and mortality in adults. The Florida Department of Juvenile Justice (FDJJ) uses the Positive Achievement Change Tool (PACT) to measure the likelihood of re-offense and assess how best to rehabilitate individuals. The nature and severity of child maltreatment, as well as the school based experiences of the juvenile offender is assessed by the PACT.

Objective: Truancy, or unexcused absences from school, is used as a predictor of delinquency – unlawful behavior – in youth. The purpose of this study is to assess the impact of ACEs on habitual truancy in juvenile offenders in the state of Florida. We hypothesized that high odds will accompany individual ACEs, and that juvenile offenders who are habitually truant will have higher odds of accumulated ACEs, relative to their peers.

Method: The FDJJ pre-screens juvenile offenders that are referred by law enforcement. More serious offenders are given a full screen. The results of the PACT screens were reported to us in a de-identified database. A victims' advocate was consulted to help determine the criteria for selecting the sample of individuals used in this study because of the size of the database. There were 71,253 individuals with full PACT screens in the sample. Felonies and misdemeanors committed by the youth include various classifications: property, personal/physical, substance abuse and criminal violations. Individuals were grouped in two ways for analysis: according to their gender and based on the number of offenses they had committed. Statistical Analysis System (SAS) was used to analyze the data and calculate the odds ratios. ACE sum is derived by an individual count of the ACEs of each offender.

Results: The sample was 20% female. Repeat offenders comprised 83.2% of the sample. Domestic violence in the home and substance abuse by a parent or guardian were the most prevalent ACEs among truant offenders, regardless of the number of offenses they have committed or their gender. Household domestic violence in offenders exhibiting truancy had odds ratios of 3.47 and 3.15 for males and females, respectively. The odds ratios of truancy and ACE sums were directly proportional, and ranged from 0.23 to 3.97; males had a wider range with odds ratios from 0.23 to 3.97, while truant females' odds ratios were from 0.20 to 2.23. Overall, males were more likely to be truant when compared to females. Habitually truant female offenders were more likely to have an ACE sum of 8 – with possible ACE sums ranging from 0 to 10 – when compared to the ACE sums of females who did not exhibit habitual truancy. Males who were truant had the highest odds of having an ACE sum of 10, also when compared to non-truant members of their group. Of those living with a biological parent and suffering from an ACE, the odds in males ranged from 0.27 to 1.07. Females' likelihood ranged from 0.28 to 1.21 when biological parents were present in the household.

Conclusion: Domestic violence was the main contributor to truant behavior in juvenile delinquents. Males were more likely to be truant. Of the habitually truant population, males had higher ACE sums than females. The presence of a biological parent in the residence served as a protective factor to both male and female juvenile offenders; the offenders had a reduced likelihood of having an ACE.

"Association of CYP2D6 gene copy number with Metoprolol response in the Pharmacogenomic Evaluation of Antihypertensive Responses 2 (PEAR2) Study"

Sofia Garcia, Julie Johnson, Pharm.D., Taimor Langaee, Ph.D. and Ben Burkley
Department of Pharmacotherapy and Translational Research

Cytochrome P450 2D6 (CYP2D6) is a major drug-metabolizing enzyme involved in the metabolism of different drug classes including: β-blockers, opioids, antidepressant and antipsychotic agents. More than 80 polymorphic variations of this gene have been identified which include copy number variants such as gene deletions, duplications and multiplications. Carriers of different copy numbers of the *CYP2D6* gene have increased or reduced ability to metabolize drugs via the CYP2D6 pathway. The functional impact of these variants alters the pharmacokinetics of drug metabolism and can result in differences in drug efficacy and/or toxicity. Drug therapy in patients with a different number of gene copies may result in treatment failure or increased toxicity. Personalizing medicine through pharmacogenetic testing is becoming necessary to address this issue. Current genotyping techniques used in the laboratory lack the ability to determine the exact number of copies of the functional gene. We focused on standardizing duplication and deletion assays to genotype patients that were enrolled in the Pharmacogenomic Evaluation of Antihypertensive Responses 2 (PEAR 2) trial, a multi-center, open-label and sequential monotherapy study. Patients of all races between ages of 18 and 65 with mild to moderate hypertension were treated with a β1-selective blocker (metoprolol) followed by a thiazide diuretic (Chlorthalidone). In this project, we tested whether there is a relationship between *CYP2D6* copy number and drug efficacy in terms of blood pressure control, heart rate and the presence of drug-related adverse events to the beta-blocker metoprolol in these patients. Statistical analysis including ANCOVA and linear regression were adjusted by baseline blood pressure, heart rate, age and sex. C*YP2D6* copy number polymorphisms and drug-related adverse events were also evaluated using Fisher's exact test and Cochran-Armitage trend test. A total of 192 patients were genotyped, 14 patients showed deletions of the *CYP2D6* gene and 10 patients showed duplications/multiplications of the gene. Results demonstrated no significant association between *CYP2D6* copy number and systolic blood pressure in Blacks (P=0.71), non-Blacks (P=0.19) and all races (P=0.18). Likewise, no statistically significant association was found between *CYP2D6* copy number variants and changes in diastolic blood pressure (Black P=0.52; non-Blacks P=0.14; all races P=0.13). In addition, no association was found between *CYP2D6* copy number and heart rate (Blacks P=0.41; non-Blacks P=0.36; all races P=0.62) or between the composite of adverse effects that included fatigue, bradycardia, dizziness, wheezing, dyspnea and *CYP2D6* copy number polymorphisms (Fisher's exact P=0.82; Trend test P=0.4). In conclusion, the results of this study showed that the number of copies of the *CYP2D6* gene do not seem to play a major role in efficacious or adverse metabolic responses to metoprolol. However, it will be necessary to repeat the analysis with a larger sample size in order to accurately establish the influence of *CYP2D6* copy number in responses to metoprolol. Ultimately, the work done in this project is a valuable asset to be included in the clinical assays that are offered at the UF Pathology Laboratories in the UF & Shands Personalized Medicine Program.

"Occlusion of the BC Artery May Lead To Apoptosis by Caspase 3".

Ashlyn Goodwin, Charles E. Wood, Ph.D.
Department of Physiology and Functional Genomics

Corticotrophin-releasing hormone (CRH) is released by the hypothalamus when the brain is put under stress from a lack of oxygen or blood. The CRH then activates the anterior pituitary gland. The anterior pituitary gland triggers the release of the adrenocorticotrophic hormone (ACTH) into the adrenal cortex to release glucocortinoids such as cortisol. Cortisol then sends the brain signals that are negative feedback. Negative feedback is needed to tell the hypothalamus to decrease the concentration of CRH and ACTH in the blood once the brain is no longer under stressful conditions. The hypothalamus controls most of the body's hormonal systems. The braciocephalic artery is an artery that supplies the brain with blood and oxygen. Occlusion of the braciocephalic artery leads to an activation of several pathways one of which is apoptosis. Caspase 3 is an enzyme that plays a key role in apoptosis, or programmed cell death. It can be triggered by mitochondria, Caspase 8 and ProCaspase. Once Caspase 3 has been activated its signals to the cell to shut down and begin execution. This study involves observing and examining the homogenized brain tissue of a fetal sheep that has been deprived of blood for ten minutes using a western blot. Western blotting is a technique used to identify and locate Caspase 3 in protein samples based on their ability to bind to specific antibodies. Western Blotting involves three steps: sample preparation, electrophoresis, and the transfer of proteins and staining (western blotting). The tissue is lysed and prepared from tissues by adding phosphate buffer. The protein concentration is determined by a Lowry Assay. The proteins in the solution are then separated by SDS page. The proteins are then transferred again by gel electrophoresis.The gel used for protein transfer is soaked in a transfer buffer and the proteins are transferred onto a membrane. The proteins stick to the membrane and a "blot" is taken of the gel. Antibodies are then added to the membrane to bind the protein of interest. There was no detection of Caspase 3 shown after running the chemiluminsecent blot. Concentrations of the samples used in this experiment may have effected the detection of Caspase 3 in the proteins during the Western Blot.

Effects of Ifnar1 Antibody in Reducing the Type I Interferon Signaling Pathway

Beulah Joseph, Michael Clare-Salzler, M.D.
Department of Pathology

Type 1 Diabetes (T1D) is a lifelong disease that results from T cell mediated destruction of pancreatic beta cells responsible for glucose metabolism through insulin secretion. Studies have shown genetic and environmental risk factors associated with T1D through cellular and molecular techniques to establish the basis for the immunpathogenesis of T1D. There has been an association between virus infection, type I interferon treatment and T1D incidence. Our mouse data also indicated that NOD, a T1D disease model, expressed enhanced interferon signaling pathway and blocking interferon receptor has decreased the incidence in NOD mice. In this research, we will characterize the effects of an interferon receptor antibody in reducing the signaling events in a β cell line, NIT cells. Current studies have found that BST2 expression is enhanced by type I interferon alpha (IFN-α), thus is used as a marker in the study. The purpose of this study is to monitor the interferon signaling pathway by detecting BST2 expression with different dosage of interferon antibody (IFNAR1).

There were two particular techniques used to monitor the interferon signaling pathway which are western blot and flow cytometry. The experiment was done on NIT cells; the antibody used was Ifnar1 antibody and its isotype IgG control. The doses used for either the antibody or IgG control were 5ug/ml, 10ug/ml, 20ug/ml, 40ug/ml, 80ug/ml and 120ug/ml. A small molecule based drug from NCI was used as a positive control.

The data analysis revealed that NIT cells respond to type I interferon characterized by increased p-stat1 expression. The Ifnar1 antibody used for this experiment was effective in inhibiting the type I signaling pathway characterized by Bst2 expression. The results showed that Ifnar1 antibody was able to inhibit Bst2 expression at all doses tested including the lowest dose 5ug/ml. The dose effects were not obvious. The IgG isotype of Ifnar1 antibody does not have inhibitive effects on Bst2 expression.

In conclusion, the lowest concentration was able to achieve inhibiting effects in IFN-α signaling pathway. The antibody could be tested further *in vivo* for effectiveness and ability in reducing T1D incidence in mice.

THE ROLE OF CORTICOTROPHIN RELEASING HORMONE IN THE AMYGDALA ON THE CARDIOVASCULAR STRESS RESPONSE IN THE SHRS

Lianette Lozada, Linda Hayward, Ph.D.
Department of Physiological Sciences, College of Veterinary Medicine

Previous research has identified that spontaneously hypertensive rats (SHRs) had a greater amount of CRH positive cells within the amygdale than the normotensive Wistar rats after undergoing AJS. The present study aimed to test the hypothesis that dysregulation of the central release of the neuropeptide, corticotrophin releasing hormone (CRH) within the central nucleus of the amygdale (CEA) contributes to exaggerated cardiovascular responses to stress in an animal model of essential hypertension. First, it was observed how SHRs and normotensive Wistar rats responded to psychological stress. Wistar male rats, SHRs and CEA-CRH receptor lesioned SHRs were exposed to 20 minutes of AJS while their cardiovascular response was being continuously measured. The CRH cells in the central amygdale of rats that underwent AJS were marked using standard inmunohistochemical processing and the cells containing CRH immunoreactivity were counted and compared between the normotensive Wistars and SHRs. SHRs showed a significantly higher blood pressure response than the Wistars to AJS. However, the heart rate response of the SHRs was mildly higher, but not significantly different, from the Wistars'. SAP variability analysis identified that the SHRs had a significantly higher normalized low frequency powers before AJS, during the AJS 1 period (minutes 2-5) and during the POST 1 period (minutes 3-6 after AJS). Heart rate variability analysis identified that before AJS and during POST1 the difference in high frequency and in the low frequency/high frequency ratio between the SHRs and Wistar rats was not significantly different. However, during AJS1 the SHRs showed a significantly higher high frequency power and a significantly lower low frequency/high frequency ratio than the Wistar rats. CEA-lesioned rats, which received bilateral microinjections of the neurotoxin saporin, to lesion CRH receptor (R1) expressing neurons, showed a more attenuated heart rate response (the delta HR was 81.2+/-16.5 in AJS1 and 7.3+/-8 .8 in POST1 for the lesioned and 106.0+/-14.9 in AJS1 and 45.2+/-5.0 in POST1 for SHRs) but a more heightened blood pressure response than SHRs during AJS (the delta SAP was 23.0+/-1.3 in AJS1 and 15.6+/-3.2 in POST1 for the lesioned and 21.4+/-1.7 in AJS1 and 5.2+/-1.7 in POST1 for the SHRs). These results imply that a greater quantity of CHR-positive neurons is not necessarily responsible for the overall heightened cardiovascular response of SHRs, however in an animal model of essential hypertension a greater amount of CRH positive cells may contribute to the higher heart rate of SHRs.

“Effect of Macrophage Migration Inhibitor Factor (MIF) overexpression on neuronal activation during stress responses’’

Lorraine M. Nieves Jurado, Mike McCowan Benedek Erdos, MD, PhD, Deborah A. Scheuer, PhD
Department of Physiology and Functional Genomics

Exaggerated blood pressure elevations caused by stress increase the risk for hypertension. Therefore, reducing the blood pressure response to stress could help to prevent the development of hypertension. Angiotensin II (AngII) is a neuropeptide that is involved in the regulation of blood pressure and in the mediation of stress- induced pressure responses. Excitatory actions of AngII on neuronal activity are mediated by AT1 receptors and the production of reactive oxygen species (ROS). In normotensive rats, AngII stimulates the production of MIF in neurons of the paraventricular nucleus of hypothalamus (PVN). MIF can scavenge ROS, and by removing ROS, it can inhibit AngII mediated blood pressure increases. In contrast, MIF expression is not stimulated by AngII in spontaneously hypertensive rats (SHRs) leading to augmented stress responses. It has been shown that activity of noradrenergic neurons in the nucleus of the solitary tract (NTS) increase during psychological stress resulting in an elevated expression of c-Fos. The focus of this project is to determine if overexpression of MIF in the PVN of SHRs can reduce stress-induced neuronal activation in noradrenergic neurons in the NTS. Adeno-associated viral vectors carrying green fluorescent protein (GFP) or MIF (AAV2-GFP or AAV2-MIF) were injected bilaterally into the PVN of 11 week-old SHRs. Three weeks later, animals were subjected to a 1-hour restraint stress followed by trans-cardiac perfusion with 4% paraformaldehyde. I performed immunohistochemistry using anti-cFos and anti-dopamine beta hydroxylase (DBH) antibodies and assesses the number of DBH positive cells and the number of double labeled, c-Fos/DBH positive cells in the NTS and Area Postrema (AP) in coronal brain section 13.80mm and 14.60mm posterior from the bregma. The number of DBH positive neurons in the AP were 63±15 in the GFP group and 43±15 in the MIF group ($p<0.05$NS). The numbers of DBH positive neurons in the NTS were 32±12 in the GFP group and 27±5 in the MIF group. The percentage of AP neurons expressing c-Fos among the DBH positive neurons was 79±15 in the GFP group and 79±17 in the MIF group. The percentage of NTS neurons expressing c-Fos among the DBH positive neurons was 80±19 in the GFP group and 73±18 on the MIF group. These results indicate that the number of DBH positive neurons in the AP declined significantly in response to overexpression of MIF in the PVN, whereas the percentage of activated, c-Fos positive noradrenergic neurons did not change significantly following MIF treatment either in the AP or NTS.

Programming human hematopoietic stem cells for generation of brown adipose tissue

Osii Mbata, Maria Grant, Ph.D., Daniel Ryder, Ph.D.
Department of Pharmacology and Therapeutics

The goal of this experiment was to test lentivirus' ability to infect human cells with the genes needed to turn hematopoietic stem cells into lipid oxidizing brown adipose cells. Lentivirus carrying rtTA (reverse transcriptase transactivator), PPARG2, CEBPB, and PRDM16, genes found in a recent study by Dr. Chad A. Cowan to differentiate mesenchymal progenitor cells derived from pluripotent stem cells into brown adipocytes, were used to infect human kidney cells. The type of human cells used was chosen for no particular reason. The infected cells were cultured and later had their RNA extracted. After RNA was transcribed to concentrated cDNA, QPCR was run to see if it expressed the desired gene. UCP1 was chosen as the target gene because it would be a tell all for this particular virus' gene transcription because it has been found in previous studies, UCP1 is expressed by brown fat tissue and promotes the oxidation of lipid and glucose. After QPCR was run it was found that UCP1 was not expressed. However, using phenotypic markers, it was found that the virus had transferred the desired genes. UCP1 was not expressed because the cells used were kidney cells and would be unable to produce due to differentiation.

The experiment is to be continued by infecting hematopoietic stem cells. If this is done successfully, the cells will be injected into obese mice to see if it will be a viable therapy for weight loss and blood glucose reduction.

Significance of Type III Virchow-Robin spaces in the brainstem in mild Traumatic Brain Injury (TBI)

Steve Noutong, Joseph Gullett, Ari Seff and David FitzGerald, M.D.*
Department of Neurology

Background and Hypothesis Mild TBI is widespread in veterans returning from Iraq or Afghanistan. Roughly 220,000 service members are estimated to have sustained an alteration or loss of consciousness while deployed. Mild TBI is associated with difficulties with memory, attention, executive function and sleep. As part of a larger study, Dr. FitzGerald noticed Type III Virchow-Robin spaces (V-R spaces) in the brainstems of young veterans who have experienced blast and been given a diagnosis of mild traumatic brain injury (mTBI). In addition, the referred type III V-R spaces appeared much larger than expected in these young (<35 years old) veterans. We hypothesized the abundance of the type III V-R spaces in these young veterans might be related to blast injuries, either through mechanical damage due to rapid flexion of the neck or by the loss of axons due to the blast and resulting Wallerian degeneration.

Aim Characterize type III V-R spaces in veterans for volume, width, length and height and compare to healthy age-matched controls for these characteristics.

Methods The sample in this study consisted of 29 Operation Iraqi Freedom and Operation Enduring Freedom (OIF/OEF) veterans who have sustained a mild Traumatic Brain Injury (with or without loss of consciousness) as a result of a blast, and 29 age-matched controls with no history of head injury or loss of consciousness. We used ImageJ, an imaging analysis software package developed and released by NIH, to characterize the VR spaces in both sets of subjects. Throughout the brainstem the V-R space were characterized as structures with a mean intensity at least three standard deviations below the average slice's intensity. Two sets of measurement were obtained. For the "Whole Measurements", suspected type III V-R spaces were included when measuring the slice's mean intensity and for the "Selective Measurements", suspected V-R spaces were excluded when measuring the slice's mean intensity.

Results The average age of veterans (n=29) and healthy controls' (n=29) groups was respectively 28.8621±0.665 years and 27.655±1.115 years. The mean value of the paired sample T test of *veterans' age-healthy controls' age* is 1.2±1.258 years (P=0.346). For the "Whole measurement", the mean volume of type III V-R spaces was found to be 7.048±1.556 mm^3 for the veterans and 4.383±1.166 mm^3. The mean value of the paired sample T test for *veteran type III V-R space's volume- hc type III V-R space's volume* is 1.206±1.258 mm^3 (P=0.403). Data collection for "Selective measurement" is still ongoing.

Conclusion Our preliminary results suggest that there is no significant difference in volume of type III V-R spaces between the two age-matched groups. In this sample of heterogeneous blast victims, these findings may indicate that after controlling for age there is no association between head injury and increased instance of Type III V-R spaces. These findings contradict a previous study in which a significant difference in the *number* of V-R spaces was found between a brain-injured group with a mean age 35 years and a control group with a mean age 20 years. However, it is likely that the failure of that group to provide *age-matched* samples resulted in spurious findings that occur as an artifact of aging in general. We believe that the method we have developed for determining the specific volume of Type III V-R spaces is an accurate calculation of brain atrophy resulting from a multitude of causes, and can pave the way for future research toward the development of specific normative values for the volume of V-R spaces that occur in normal aging.

Renal Tubule Protein Profile in Chronically Vasodilated Rats

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Maternal hemodynamic adaptations are needed to ensure healthy pregnancy. These include peripheral vasodilation, decrease in blood pressure, increase in plasma volume, and increased glomerular filtration rate (GFR) and sodium retention. These studies will test the underfill hypothesis of pregnancy, which proposes that the mother undergoes a primary vasodilation and a secondary increase in renal sodium retention to increase plasma volume in order to refill vasculature. We have previously found that many of the renal and hemodynamic adaptations that occur in pregnancy can be induced in virgin rats by chronic systemic vasodilation. Specifically, midterm pregnancy in the rat is associated with plasma volume expansion and hemodilution as well as increases in medullary phosphodiesterase 5 A (PDE5A). PDE5A is an enzyme that degrades cGMP, the second messenger of the natriuretic agents nitric oxide (NO) and atrial natiuretic peptide (ANP). This prevents the kidney from excreting sodium in response to these agents. Further, we found increases in the α-subunit of the epithelial sodium channel (ENaC), the rate-limiting subunit for channel formation, and the water channel aquapourin-2 (AQP2). These channels facilitate sodium and water retention in the collecting duct and likely contribute to volume expansion. We have further reported that 14 days of chronic vasodilation in the virgin female rat recapitulates all the alterations seen in pregnancy, listed above. These data taken together support the underfill hypothesis of pregnancy which implies that a primary vasodilation drives the secondary renal sodium and water retention and consequent volume expansion. Since sodium retention is closely regulated by the renin-angiotensin-aldosterone-system (RAAS) we have conducted a follow up study to determine whether RAAS plays a role in the volume expansion. We used Enalapril, an angiotensin converting enzyme (ACE) inhibitor to block angiotensin II (AII) formation. Enalapril also prevents breakdown of bradykinin a powerful vasodilator, and 14 days administration leads to a progressive fall in blood pressure (BP). In this case, however, there were no changes in plasma volume. The aim of the current work is to examine the collecting duct profile of the apical sodium and water transporters in the enalapril study. We measured by western blotting the protein abundance of α- β- and γ-ENaC subunits, and AQP2 from homogenates of kidney cortex, outer medulla, and inner medulla. We found that although Enalapril produced systemic vasodilation it did not change the abundance of ENaC subunits or AQP2 in any of the kidney regions. This work suggests that the collection duct modifications promoting volume expansion in response to vasodilation requires an intact RAAS.

"Effects of muscle stretching on endothelium-dependent vasodilation and endothelial nitric oxide synthase expression in rat skeletal muscle arterioles"

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Background and significance: Stretching exercise is often prescribed in patients with restricted mobility in order to prevent muscle atrophy. Studies have demonstrated that traditional stretching exercises enhance vascular endothelial function, as assessed by flow-mediated dilation in patients with ischemic heart disease; however, it remains unknown whether stretching exercise alters on the production of nitric oxide (NO) and/or endothelial nitric oxide synthase (eNOS) expression in vascular endothelial cells.

Hypothesis: We hypothesized that regular stretching of skeletal muscle in the lower limb will enhance eNOS protein levels, resulting in the improvement of NO-dependent vasodilation in skeletal muscle arterioles from aged Fischer 344 rats.

Methods: We divided twenty-four aged Fischer 344 (18 months) rats into three groups: a control group which remained sedentary in cages, a sham-stretching group, and a group which underwent static stretching of the lower for thirty minutes, five days a week, for 3 weeks. The soleus muscle of one hindlimb was maintained in a stretched position by applying a splint that maintained the ankle joint at a 30° angle for 30 minutes. At the end of the three week program of daily stretching, arterioles were isolated from both the stretched and unstretched soleus muscles of the splinted rats, and from the soleus muscles of the cage control and sham-splinted rats. Endothelial responsiveness to acetylcholine, and smooth muscle responsiveness to the NO donor, Dea-NONO-ate were evaluated in cannulated, pressurized arterioles. Arterioles were also saved at -80°C and immunoblot analysis was used to evaluate eNOS protein levels.

Results: Endothelium-dependent vasodilation to acetylcholine was increased by daily stretching. Responsiveness of the smooth muscle to NO was not altered by daily stretching. Analysis of ENOS protein is pending.

Conclusion: These data indicate that daily stretching enhances endothelial function of skeletal muscle arterioles in the hindlimb of aged rats. Changes in ENOS protein levels or activity may underlie enhanced endothelial function. More study is needed to determine whether enhanced endothelium-dependent function of skeletal muscle arteries can contribute to improvements in muscle blood flow and functional capacity in the elderly.

ACE2 activation ameliorates endothelial function in the right ventrticles of pulmonary hypertensive rats

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Background & Significance: Pulmonary hypertension is a dangerous condition that is characterized by unusually high blood pressure in the pulmonary circuit. The increase in right ventricular afterload results in significant hypertrophy that can end in right heart failure; however, the mechanisms that contribute to the hypertrophy and failure remain largely undefined. Recent discovery of the beneficial arm of the renin-angiotensin-system and the enzyme, angiotensin-converting enzyme 2 (ACE2), which converts Angiotensin-II to Angiotensin-(1-7) has led to investigation of ACE2 activators as therapeutic agents that may be able to improve lung and heart function in pulmonary hypertension. Impaired function of the resistance vessels that regulate blood flow to the right ventricle could contribute to right ventricular failure; therefore, we investigated the effects of experimental pulmonary hypertension on endothelium-dependent vasodilation of right ventricular resistance arteries. We also investigated the possibility that the ACE2 activator, diminazene aceturate (DIZE), ameliorates endothelial function of resistance arteries in the right ventricle of rats with experimental pulmonary hypertension.

Hypothesis: DIZE treatment will ameliorate endothelial function of resistance arteries isolated from the hypertrophied right ventricle of rats with experimental pulmonary hypertension.

Methods: 8week old Sprague Dawley rats were divided into four groups: control (no treatment), monocrotaline-treated (MCT) to induce pulmonary hypertension, monocrotaline-treated (MCT) + DIZE, and DIZE alone (no MCT treatment). DIZE was injected daily for 4 weeks. After 4 weeks of treatment, right ventricular systolic pressures were measured (to assess for relative pulmonary pressure) in anesthetized animals and then the hearts were excised to obtain resistance arteries from the right ventricular free wall. Endothelium-dependent and -independent vasodilation were assessed by assessing responses to increasing concentrations of acetylcholine and the nitric oxide donor, Dea-NONO-ate, respectively.

Results: Endothelium-dependent vasodilation is impaired in right ventricular resistance arteries from MCT-treated rats as compared to those from control rats. In contrast, responses to exogenous nitric oxide were not altered in right ventricular resistance arteries from MCT-treated rats. Preliminary results indicate that DIZE treatment restores responsiveness to acetylcholine in right ventricular resistance arteries from MCT-treated rats. These data suggest that pulmonary hypertension impairs endothelial function of the resistance vasculature in the right ventricle. Activation of the protective axis of the renin-angiotensin system may correct this loss of endothelial function in right ventricular resistance arteries of rats with pulmonary hypertension. Future work will focus on determining whether DIZE-induced correction of endothelial dysfunction in the resistance vasculature contributes to improved perfusion of the right ventricle in experimental pulmonary hypertension.

The Effects of Viral Proteins Serp-1 and M-T7 on Gene Expression in Mononuclear Blood Cells Isolated from Patients with Chronic Transplant Vasculopathy

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OBJECTIVE: To treat mononuclear blood cells isolated from patients after cardiac transplant in the presence of Serp-1, M-T7, or saline and use SYBR Reverse Transcriptase Polymerase Chain Reaction (PCR) array to analyze the changes in gene expression.

BACKGROUND: Approximately 85,000 heart transplants have been performed since 1982. The development of immunosuppressive therapies has greatly improved the prognosis of heart transplant patients and reduced risk of acute antibody-mediated transplant rejection. However, chronic rejection still remains a significant cause of morbidity in patients in the first year after allograft transplant. The known causes of chronic allograft rejection are diffuse arterial plaque growth, obstruction to blood flow, and scarring of the transplanted organ. Treatment of chronic rejection has been limited to the use of cholesterol lowering agents, statins, treatment of recurrent episodes of acute rejection, and in severe cases re-transplantation. Anti-inflammatory agents may hold promise in the treatment and prevention of cardiac allograft vasculopathy. Specifically, the Lucas lab has developed a new class of virus-derived serine protease inhibitors (serpins) such as Serp-1 and chemokine modulating proteins like M-T7 as therapeutics. Changes in gene expression of peripheral blood mononuclear cells (PBMCs) may provide insight into how these viral proteins may effectively reduce inflammatory cell response. These analyses will assess the potential for therapeutic use of these proteins in patients to prevent chronic transplant vascular disease.

METHODS: Blood from patients with transplant vasculopathy is treated with Saline, Serp-1, or M-T7. Then the total RNA is isolated. The RT-PCR array gene expression data of cells treated with either Serp-1 or M-T7 is compared to saline-treated from the same patient. This entire dataset is then compared to non-vasculopathy patient blood, to pinpoint vasculopathy specific Serp-1 and M-T7 mediated changes. Genes of interest will be noted based on their fold changes in expression.

RESULTS: We hypothesize that Serp-1 and/or M-T7 will have an effect on gene expression. Patient blood has been collected, RNA isolated, and cDNA synthesized. Optimization of the PCR array is currently in progress.

A PHARMACOEPIDEMIOLOGICAL APPROACH TO THE USE OF THE BRADFORD HILL CRITERIA OF BIOLOGICAL PLAUSIBILITY IN PHARMACOTHERAPY, AND ITS APPLICATION IN ESTABLISHING A CAUSAL RELATIONSHIP BETWEEN DRUG ADMINISTRATION AND ADVERSE DRUG EVENTS

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A literature review conducted to assess the use and various definitions of The Bradford Hill criteria of biological plausibility among different publications. The literature review intends to create a clear and concise view into the definition and use of the Bradford Hill criteria of biological plausibility in today's medical research. The purpose of this goal is to provide a concrete set of guidelines, which can provide a useful research tool for the assessment of biological causation, with particular focus on its application within pharmacoepidemiology, pharmacotherapy, and drug development. As part of the applications of the literature review, it also assesses the application of The Bradford Hill criteria of biological plausibility in establishing a causal relationship between drug administration and adverse drug events.

The determination of the causal association of different xenobiotics –the relationship between a presumptive outcome as a result of the administration of a particular xenobiotic- needs to be consistent with existing physiological and biological principles.

The focus and conclusion from the 37 articles selected for this literature review have 2 main parameters into account. Both parameters, pharmacodynamic and pharmacokinetic principles, are used to determine the biological plausibility between the administration of a xenobiotic and an outcome –whether it be the desired effect or adverse drug event.

Within the three main sub-parameters (metabolism, site of action, and mechanism of action) the metabolism of the xenobiotic is fundamentally the most practical when determining the outcome after the administration of a xenobiotic. The above is particularly true for adverse drug events as the mechanism and site of action only account for the physiological effects of the xenobiotic administered. On the other hand, utilizing the metabolism of the xenobiotic as a cause for the outcome observed or expected has into account the effects of the parent compound administered, as well as the whole cascade of metabolites produced in the biotransformation and final excretion or bioaccumulation of the xenobiotic from the body.