Program and Abstract Booklet

Combined Meeting of the Iowa and Nebraska Physiological Societies

Chapters of The American Physiological Society

Des Moines University
October 21–22, 2011
The Iowa and Nebraska Physiological Societies would like to take this opportunity to gratefully acknowledge the following contributors for their support of the 2011 Combined Meeting of the Iowa and Nebraska Physiological Societies:

The American Physiological Society

The American Physiological Society is proud to be the major sponsor of the 2011 Combined Meeting of the Iowa and Nebraska Physiological Societies.

University of Nebraska Medical Center
Department of Cellular and Integrative Physiology

We would like to thank Pearl Sorensen, Janine Wilson, and Debra Davis from the Department of Cellular and Integrative Physiology at the University of Nebraska Medical Center for their administrative help in organizing the meeting.

Thank you for your support!
Welcome Address

Welcome to Des Moines for the 2011 Combined Meeting of the Iowa and Nebraska Physiological Societies! We’re pleased you’ve joined us and hope you will have the chance to see some old friends, make new friends, and forge some new professional relationships. The intent of this meeting is to bring together scientists and students from Iowa, Nebraska, and surrounding states to foster networking, provide educational opportunities, and offer a venue for the presentation of scientific findings and ideas related to the field of physiology.

Starting on Friday evening, we have a buffet dinner planned along with special music from the Des Moines University Chamber Orchestra. We hope you will join us to enjoy food, music, and fellowship with physiologists from Iowa, Nebraska, and surrounding states.

On Saturday, we have an outstanding scientific program lined up for physiologists at all levels. Our keynote research speaker, Dr. Karie Scrogin, from Loyola University in Chicago, will present a talk entitled “New Tricks for an Old Molecule: Discovery of a Novel Role for Serotonin in the Neural Control of the Circulation” which should provide excellent integrative appeal to physiologists working at all levels of our field. Our keynote education address by Dr. Arri Eisen from Emory University in Atlanta promises to be interesting and exciting as he talks about his journey in teaching in a talk entitled “Freshmen, Post-Docs and Monks: My Journey into Learning How to Teach.” We also have two exciting presentations from “local” physiologists on the agenda. Dr. Keshore Bidasee from the University of Nebraska Medical Center will give a talk on “Cellular Heterogeneity in Cardiac Ryanodine Receptor Function During Diabetes” while Dr. Mike Lyons from Kirkwood Community College will speak on “Undergraduate Research in Two and Four Year Institutions: How to Develop, Implement, and Assess Research Experiences for First-time Physiology Students.” To round out our list of presentations, we have two graduate student research talks scheduled, one from each society, chosen based on the scientific merit of their abstracts. Finally, there will be numerous posters on display throughout the day that will be presented and judged in the afternoon. An award ($1000 to attend Experimental Biology meeting in 2012) will be given for the best overall undergraduate and graduate research posters/presentations. There will also be a non-monetary award given for the best non-research poster/presentation.

Three concurrent workshops are another highlight on our agenda this year. These have been developed with the interests of college/university faculty, high school teachers, and undergraduate/graduate students in mind. Drs. Mark Chapleau and Clay Peterson from the University of Iowa will conduct a computer technology session for college/university faculty while Dr. Gina Schatteman, also from the University of Iowa, will hold a teaching workshop for high school teachers dealing with curriculum supplements developed by the National Institutes of Health. Finally, a panel of local and national experts has been pulled together for our students to discuss “alternative” biomedical careers related to physiology.

We hope you will take time to visit our vendor displays that include booths by ADInstruments, DSI, and Kent Scientific. The success of this meeting would not be possible without the financial support of our sponsors.

We appreciate that you have made the trip to Des Moines to take part in this combined meeting. You are very important to the success of the meeting and your society. We hope you find this year’s meeting insightful, productive and enjoyable!

Julia Moffitt, Ph.D.  
President, Iowa Physiological Society  

G. Patrick Lambert, Ph.D.  
President, Nebraska Physiological Society
2011 Combined Meeting of the Iowa and Nebraska Physiological Societies

Des Moines University
Olsen Medical Education Center
3200 Grand Avenue
Des Moines, IA

October 21-22, 2011

Friday, October 21
4:00 – 6:00 pm  Registration open in Lobby of the Olsen Medical Education Center
6:30 – 9:00 pm  Social Function – Buffet dinner with music at the Olsen Medical Education Center

Saturday, October 22
7:45 – 8:45 am  Breakfast, Registration, Poster set-up, Judging information distributed
8:45 – 9:00 am  Opening remarks: Julia Moffitt, PhD, Des Moines University, President, Iowa Physiological Society; G. Patrick Lambert, PhD, Creighton University, President, Nebraska Physiological Society
9:00 – 10:00 am  NPS Keynote Education Address – Arri Eisen, PhD, Emory University: “Freshmen, Post-docs, and Monks: My Journey into Learning How to Teach”
10:00 – 10:15 am  Break/Exhibitor Booths/Poster viewing
10:15 – 10:45 am  NPS Research Address - Keshore Bidasee, PhD, University of Nebraska Medical Center: "Reactive Carbonyl Species: A New Player in the Pathogenesis of Diabetic Heart Failure"
10:45- 11:00 am  Nebraska Physiological Society Oral Presentation - Erin Rosenbaugh “Neuronal Uptake and Subcellular Localization of Nanoformulated Copper/Zinc Superoxide Dismutase”
11:00 – 11:15 am  Iowa Physiological Society Oral Presentation - Katrin Hollinger “PGC1-alpha Over-expression Rescues Dystrophin Deficient Skeletal Muscle”
11:15– 12:45 pm  Concurrent Workshops
  - College/University Teaching Workshop (Chair: Harald Stauss, MD, PhD, University of Iowa) Munroe Building 101
    o  Speakers:
      ▪  Mark Chapleau, PhD, University of Iowa: Computer Simulation
      ▪  Clay Peterson, PhD, University of Iowa: Technology in Teaching
  - High School Teaching workshop (Chair: Gina Schatteman, PhD, University of Iowa) Munroe Building 102
    o  Working with the National Institutes of Health Curriculum Supplements (http://science.education.nih.gov/)

October 21-22, 2011
What's new in anatomy and physiology
- Undergrad/Grad/Post-Doc Panel Discussion - Alternative Career Opportunities
  (Chairs: Julia Moffitt, PhD, Des Moines University; G. Patrick Lambert, PhD, Creighton University) Olsen Medical Education Center
  - Panelists:
    - Kelly Pitts, PhD, Corgenix Medical Corp., Chair APS Physiologists in Industry Committee
    - Darin Gylten, BA, MA, RN, Cardiac Rehabilitation, University of Iowa Hospitals and Clinics
    - Jack Johnson, BA, MS, General Manager, Aspen Athletic Club
    - Meg Robison, Medical Writer, Robison & Associates Medical Communications, LLC
    - Mike Dixon, UNeMed Corporation, University of Nebraska Medical Center
    - Lisa Vroegh - Des Moines University Admissions

12:45 – 1:30 pm Lunch and “News from the Chapter Advisory Committee”
  (Harald Stauss, MD, PhD, University of Iowa)

1:30 – 2:30 pm IPS Keynote Research Address: Karie Scrogin, PhD, Loyola University: “New Tricks for an Old Molecule: Discovery of a Novel Role for Serotonin in the Neural Control of the Circulation”

2:30 – 4:30 pm Poster viewing and Competition (competition for undergraduate and graduate posters only)

4:30 – 5:00 pm IPS Education Address – Mike Lyons, PhD, Kirkwood Community College: “Undergraduate Research in Two and Four Year Institutions: How to Develop, Implement, and Assess Research Experiences for First-time Physiology Students”

5:00 – 5:30 pm Poster awards and closing remarks – Drs. Moffitt and Lambert

5:30– 6:00 pm IPS and NPS Business Meetings - Munroe 101 & 102 for Business Meetings
Nebraska Physiological Society Educational Keynote Speaker

Arri Eisen, Ph.D.
Emory University

Arri Eisen received his BS with Honors in Biology from the University of North Carolina, Chapel Hill, and then his PhD in Biochemistry from the University of Washington, Seattle. He has been teaching and involved in science education at Emory since 1990—teaching science courses to undergraduate and graduate students, post-doctoral fellows, and faculty and leading courses in how to teach more effectively for grad students, post-doctoral fellows, and faculty.

In addition to coordinating the How to Teach seminar for the NIH-sponsored Fellowships in Research and Science Teaching program for postdoctoral fellows for the past decade and organizing teaching mentors for fellows at the Atlanta University Center, he was the PI and helped administer an NSF Research Experience for Undergraduates and the Howard Hughes summer research programs for a decade, working especially to increase women’s and minorities’ participation in science. In Biology, Dr. Eisen developed a two-semester, investigative Honors Introductory Biology course at Emory that he taught for 12 years; he has also taught undergraduate seminars in Genetically Modified Organisms, Viruses, the Origin of Life, and Evolution, as well as courses in Cell Biology, Developmental Biology, and Experimental Cell and Developmental Biology. In the Institute in the Liberal Arts, he teaches and develops programs in Science and the nature of evidence, science and religion and science and ethics. In the Center for Ethics, he also teaches and studies research ethics education. Dr. Eisen has co-authored an introductory biology text, The Living Staircase, and publishes in peer-reviewed journals with graduate and graduate student co-authors in both basic research (genetic regulation in yeast and Drosophila) and in education. Since 2006, in collaboration with the Dalai Lama and the Emory Tibet Partnership, Dr. Eisen has helped lead the Emory Tibet Science Initiative, teaching science to monks and nuns in Dharamsala, India, and writing textbooks for this project the goal of which is to integrate modern science into the curriculum of all Tibetan Buddhist monks and nuns in India. He has given presentations on innovative teaching at Emory and across the country, including at GA Tech, the University of Nebraska-Lincoln, and at the National Science Teachers Association and the Association of Biology Laboratory Educators meetings.
Keshore R. Bidasee is Associate Professor (with tenure) and Vice Chair-Graduate Education, Department of Pharmacology and Experimental Neuroscience (PEN), University of Nebraska Medical Center (UNMC), Omaha, NE. Born in San Juan, Trinidad, West Indies, he attended the University of the West Indies, St Augustine, Trinidad, where he earned his B.Sc (1986) and Ph.D (1991) in Analytical Chemistry. In summer of 1991, he joined the laboratory of Dr. Henry R. Besch, Jr., Department of Pharmacology and Toxicology Indiana University School of Medicine as a postdoctoral fellow to conduct structure-activity relationship studies on the plant alkaloid ryanodine, a modulator of intracellular ryanodine receptor. In 1994 he was promoted to Instructor and in 1997 Assistant Professor (p/t) in the Department of Pharmacology and Toxicology, Indiana University School of Medicine. In 2000 he received his first R01 award to study the role ryanodine receptors in diabetic cardiomyopathy. Two years later he moved to the then Department of Pharmacology, University of Nebraska Medical Center, Omaha NE as an Associate Professor.

Dr. Bidasee has 42 original research publications, is a regular member on an NIH study section, an APS member and was elected a councilor of the Nebraska Physiological Society. His lab is funded by the National Institute of Health and the Nebraska Redox Biology Center to study the effects of carbonylation (a type of post-translational modification) on the function of Ca2+ cycling protein during diabetes. Edna Ittner Pediatric Foundation and the American Diabetes Association also provided research funding for his research. Dr. Bidasee is also involved in graduate and professional teaching at UNMC. He is course director for Receptors and Cell Signaling (PHAR-901), and teaches sections in several team-taught courses. He is also a member of UNMC Graduate Affairs Committee and a Vice Chair of UNMC Institutional Animal Care and Use Committee.
Dr. Karie Scrogin earned her undergraduate degree in Biology at Lewis and Clark College in Portland, Oregon and thereafter completed a Ph.D. in Behavioral Neuroscience at Oregon Health Sciences University in 1992. While at OHSU she began studying the effects of stress and dietary salt intake on the neural control of circulation. After graduating, Dr. Scrogin obtained an Alexander Von Humboldt research fellowship to study autonomic mechanisms of hypertension in the laboratory of Dr. Frederich Luft in Erlangen, Germany. A year later, Dr. Scrogin moved with Dr. Luft to established a new laboratory at the Max Delbruch Center for Molecular Medicine in the former East German section of Berlin. In 1995, Dr. Scrogin moved back to the US to work with Dr. Kim Johnson in the Cardiovascular Research Center at the University of Iowa where she began her own line of research examining the role of central serotonin in blood pressure regulation. She was subsequently recruited to the faculty at Loyola University Chicago, Stritch School of Medicine in 1999. She is currently a professor in the Department of Molecular Pharmacology and Therapeutics at Loyola. She continues to pursue research on serotonin and blood pressure control but has expanded her research efforts to study depression in heart failure. In addition, Dr. Scrogin directs the Systems Biology core course for graduate students and teaches autonomic pharmacology to 2nd year medical students. She has been elected to various positions in regional and national scientific societies including the Society for Neuroscience, the American Physiological Society as well as the American Society for Pharmacology and Experimental Therapeutics for which she recently served as President of the local Great Lakes Chapter.
Michael Lyons is a professor of human physiology and anatomy and Kirkwood Community College in Cedar Rapids, Iowa. He is also an adjunct assistant professor with the Health and Human Physiology Department at the University of Iowa. Dr. Lyons earned a masters degree in biology from Drake University and a Ph.D. in Science Education with a research focus on the effects of inquiry laboratories on student learning and critical thinking processes. His research has led to the creation of a Human Physiology class that culminates in a student-driven original research project to facilitate not only an understanding of science, but develop improved critical thinking and analytical skills. The positive results have led Dr. Lyons to promote a pedagogical shift towards student research for all undergraduate physiology classes.
Undergraduate
Posters
U1 - U14
HYPOXIA INCREASES HIF-1 ALPHA AND GLUTAMATERIC NMDA RECEPTORS IN NEURONS

Craig J Cunningham, Neeru M Sharma, Hong Zheng, and Kaushik P. Patel, Department of Cellular and Integrative Physiology, University of Nebraska Medical Center, Omaha, NE.

During chronic heart failure (CHF) blood supply to the brain may be compromised. A relative shortage of blood results in an oxygen deficiency, hypoxia. Previously we have shown an increase in NMDA, NR1 receptors in the paraventricular nucleus (PVN) during CHF. In the present studies we have shown an increase in mRNA and protein for Hypoxia-Inducible-Factor 1 alpha (HIF-1 alpha), NMDAR1 (NR1), and NMDAR (NR2B 2B) during hypoxia in a neuronal cell line (NG108-15). Real-time PCR showed significant increase of HIF-1 alpha mRNA at 4 and 8 hours (4h = 1.314±0.019 vs. 1±0; 8h = 1.423±0.12 vs. 1±0). Consistent with mRNA, western blot confirmed approximately 2-fold increase in HIF-1 alpha protein levels at 12h. Hypoxia treatment also showed significant increase in NR1 mRNA at 4h (6.5 fold) and 8h (7.2 fold). Moreover, western blot confirmed a significant increase in NR1 protein levels after 12h of hypoxia (1.425±0.230 vs. 1±0). Furthermore, we observed increased immunostaining of HIF-1 alpha and NR1 in neuronal cells exposed to hypoxia for 12h. NR expression also 2B showed an increasing trend in mRNA and protein. Taken together we conclude that hypoxia induces enhanced expression HIF-1 alpha which forms a complex with constitutively expressed HIF-1ß to form a heterodimeric transcription factor that promotes transcription of NR1 and NR resulting in up-regulation of NR1 and NR 2B 2B protein in NG108 cells.
LOCALIZATION OF THE CALCIUM-SENSING RECEPTOR AT THE VERTEBRATE NEUROMUSCULAR JUNCTION

Jamie Morford and Clark Lindgren, Grinnell College, Grinnell, IA.

The calcium-sensing receptor is a G-protein coupled receptor that was first identified in parathyroid cells. More recently it has been found in neurons and various glial cells of the central nervous system. Here we use western blot analysis and immunofluorescence to localize the calcium-sensing receptor to the nerve terminal and possibly the perisynaptic Schwann cells of the neuromuscular junction.
The most common side effect of statins is skeletal muscle myopathy, which is more likely in exercisers. We investigated the interaction of statin treatment with novel vs. accustomed exercise on muscle function and heat shock protein (Hsp) expression. We hypothesized that exacerbation of statin-induced muscle dysfunction by exercise is specific to untrained muscle, while prior training protects against losses in muscle function. Within the Hsp family, Hsp25 and aB-crystallin are both up-regulated with exercise training and are associated with maintenance of muscle integrity after damaging contractions. Mice received daily atorvastatin (15 mg/kg) or placebo for 2 wks, with/without voluntary wheel running (RW) (Novel & Sedentary groups). Accustomed groups completed 3 days of RW prior to placebo or statin treatment (n= 6-7/group). In vivo plantarflexor isometric force and fatigability were measured with a dual mode lever system, and Hsp25 and aB-crystallin muscle protein levels were quantified with Western blot. Statin treatment reduced RW activity on days 1-3 compared to placebo in novel groups (p<0.05). RW activity was not different between statin and placebo in accustomed groups. Statin treatment reduced force in sedentary and novel-exercise groups compared to placebo (21 ±2% and 35±4%, respectively, p<0.05), while accustomed exercise prevented statin-associated force loss. Fatigability was the same among groups. Hsp25 and aB-crystallin levels were increased 2- to 9-fold with novel and accustomed RW compared to sedentary, independent of statin treatment (p<0.05). These results suggest that as little as 3 days of exercise prior to statin treatment can protect against muscle dysfunction rather than exacerbate it, as seen with novel exercise; and exercise-induced Hsp up-regulation may contribute to this protective effect.
EFFECTS OF GLUTAMINE SUPPLEMENTATION ON MUSCLE FUNCTION IN A MOUSE MODEL OF SPINAL CORD INJURY

Carissa Chamney, Ethan Garrigan, Michelle Godar and Kimberly Huey, Drake University College of Pharmacy & Health Sciences, Des Moines, IA.

Spinal cord injury (SCI) results in loss of muscle function due to rapid breakdown of contractile proteins and inflammation that hinder rehabilitation. Glutamine plays a critical role in muscle integrity and reducing inflammation, but impaired synthesis occurs under stressful conditions. Glutamine supplementation improves clinical outcomes, but its effects on skeletal muscle in the early stages after SCI are unknown. Improvements with glutamine in non-skeletal muscle tissues have been related to increased heat shock protein 70 (Hsp70) and Hsp25, but the muscle response may differ since it is the largest contributor to plasma glutamine. These experiments tested the hypothesis that glutamine preserves muscle function in a mouse SCI model and this is associated with increased Hsp expression. Changes in muscle mass, plantarflexor isometric force, fatigability, and muscle levels of Hsp70 and Hsp25 protein (Western blot) were measured 7 days after sham or spinal cord transection (ST) surgery in mice receiving placebo or glutamine (n=7-8/group). ST reduced gastrocnemius mass independent of placebo or glutamine (p<0.05). Maximal isometric force normalized to body mass was not different among groups, but glutamine prevented the increased fatigability with placebo in ST compared to Sham (59±4 vs. 34±4% of initial force after 10 contractions, respectively, p<0.05). Glutamine was associated with decreased Hsp70 and Hsp25 with ST compared to placebo (49±3 and 44±5% of placebo, respectively, p<0.05). Functionally, early increases in fatigability after SCI can be reversed with glutamine. ST-associated reductions in Hsp70 and Hsp25 with glutamine compared to placebo suggest lower stress in the muscle, possibly related to a reduced necessity to produce endogenous glutamine. These findings support glutamine as a therapeutic intervention to accelerate the recovery of muscle function after a SCI.
Systemic lupus erythematosus (SLE or lupus) is a chronic debilitating autoimmune disease that ultimately leads to tissue destruction due to autoantibodies and interferon activation. Previous studies have demonstrated that lupus has a strong genetic basis. In this study we tested to see if a lupus-associated single nucleotide polymorphism (SNP) in the gene PXK affects the expression of the cis gene product on a mRNA level. gDNA and cDNA were tested using a TaqMan allelic discrimination assay with real-time PCR technology. The significance between the alleles was calculated using the Wilcoxon Match-Pairs Signed Rank Test. This study found that the A allele in the SNP rs 7610449 was found to be over-expressed compared to the G allele at a statistically significant level ($p = 0.041$). The results of this study give a possible biological relevance to the statistical association of rs 7610449 with lupus. The ultimate goal of these studies is to define the genetic and biological etiology that can be used to develop therapeutics aimed at altering targetable inflammatory pathways in lupus patients.
N-ACETYLASPARTYLGLUTAMATE MODULATES NICOTINE INDUCED Nitric Oxide RELEASE AT LIZARD NEUROMUSCULAR JUNCTION

Xingjie Zhang, Elaine M. Marzluff and Clark A. Lindgren, Grinnell College, Grinnell IA.

N-acetylaspartylglutamate (NAAG), the most abundant neuropeptide in the mammalian CNS, has been found to play important roles in pain, familial ALS, and schizophrenia, but its exact physiological role is unclear. We studied the effect of NAAG on the synthesis of nitric oxide (NO) at the neuromuscular junction (NMJ) of the lizard, Anolis carolinensis. In order to accurately measure NO levels in real time, we developed a novel technique that utilizes carboxy-PTIO and mass spectrometry to monitor NO levels in the perfusate bathing lizard NMJs. Using this technique we found that, in contrast to results obtained using the fluorescence NO probe DAF-FM, application of exogenous NAAG by itself does not trigger NO release at the NMJ. In contrast, it is observed that when the hydrolysis of NAAG is inhibited, the application of NAAG inhibits nicotine induced NO release. This result suggests that NAAG plays a modulating role in the nicotine induced NO release.
EFFECTS OF COGNITIVE STRESS ON PHYSIOLOGICAL PROCESSES IN DIVISION 1 COLLEGE ATHLETES COMPARED TO NON-ATHLETES

Katie Schechinger, Rachel Bump, Ronald Torry, Rhonda Beemer. Ellis Disease Prevention Lab, Drake University, College of Pharmacy and Health Sciences, Des Moines, IA.

A recent study showed that, based on cortisol titers, elite athletes demonstrate less of a response to cognitive stress challenge than non-athletes and the authors attributed this to better coping mechanisms in elite athletes (Conzelman, et al., 2010). Although cortisol is a reliable measurement of stress response, it cannot assess acute changes in stress and titers can be affected by many variables including food, time of day, and hormones. The purpose of this study was to measure, in real-time, noninvasive physiological responses to cognitive stress tasks in athletes compared to non-athletes. Heart rate (HR) and skin temperature (ST) changes were continuously measured in 13 subjects (6 Division-1 athletes, 7 non-college athletes) during a color Stroop test and an arithmetic test; two cognitive stressors (Kennedy and Scholey, 2000). Each cognitive exam lasted two minutes followed by a recovery period. Baseline resting HR was significantly lower in athletes (65.8±6.2 bpm) compared to non-athletes (77.5±9.5 bpm; p=0.03). Each cognitive stress period significantly increased HR from baseline (Stroop: athletes 3.7±3.4%; non-athletes 3.9±2.2%; Math: athletes 7.4±4.0%; non-athletes 7.7±1.9%). However, neither the absolute change nor the percentage change in HR were significantly different between groups. Although athletes tended to have a higher overall ST, there was no significant difference between groups in absolute ST or change in ST during the stress periods. Factoring in gender as a covariate did not alter the HR or ST results. Speed (Stroop = 119.5±11.5 words/2min; Math = 24.2±11.1 calculations/2min) and accuracy (Stroop = 98.2±2.5% correct; Math = 88.1±11.7% correct) on the stress tests were similar between athletes and non-athletes. These results suggest that the coping mechanism for cognitive stress does not differ between college athletes and non-athletes.
REduced transtubular K concentration gradient (ttkg) in the distal nephron of BK-β4 knockout mice (β4ko) on Na deficient, high K diets

Kari Echtenkamp, Lori. I. Hatcher, Ryan, B. Cornelius, Steven C. Sansom, University of Nebraska Medical Center, Omaha, NE.

The BK-β4 subunit is localized with the BK-α (pore-forming) subunit in the apical membrane of the intercalated cells of the distal nephron. β4KO exhibit deficient Na-independent K secretion, as determined by renal K handling when fed a Na deficient, high K diet; however, the mechanism for this defect is not understood. These experiments were designed to determine whether the deficiency in K secretion is due to a reduced electrical gradient across the distal nephron or failure to increase distal flow that mitigates the lumen to plasma K concentration gradient. We used C57Bl6 mice and metabolic cages to assess renal outputs of K, Na, osmolality and urea. We determined the magnitude of the electrical gradient for K by calculating the transtubular K gradient (TTKG) for wild type (WT) on a control or high K diet (HK) and WT and β4KO mice fed a low Na, high K diet (LNaHK). The TTKG is the concentration gradient for K across the cortical distal nephron before the urine is concentrated by the extraction of water. We found that the TTKG increased significantly (p<0.05), from 10.5 ±0.5 (mean SEM) in control to 24.2 ±1.0 in HK WT. TTKG was not different in LNaHK WT (25.6 ±2.5), compared to HK WT. However, TTKG was significantly less (14.9 ±1.2) in LNaHK β4KO, compared with LNaHK WT. The urinary flows and the calculated urea concentrations were not different between LNaHK WT and LNaHK β4KO. We conclude that the defective Na-independent K secretion in β4KO is the result of a reduced electro-negative transtubular potential in the distal nephron.
LOCALIZATION, PACKAGING, AND UTILIZATION OF N-ACETYLASPARTYLGLUTAMATE AS A NEUROTRANSMITTER AT THE LIZARD NEUROMUSCULAR JUNCTION

Kathryn Walder¹, Steve Ryan¹, Tomasz Bzdega², Rafal Olszewski², Joseph Neale², Clark Lindgren¹, ¹Department of Biology, Grinnell College, Grinnell, IA, ²Department of Biology, Georgetown University, Washington DC.

This study establishes the presence of N-acetylaspartylglutamate (NAAG) at the lizard neuromuscular junction and shows its potential functionality through receptors for NAAG as well as its hydrolyzed product, glutamate. We used immunofluorescence costained with AM1-44 (for synaptic vesicles), Dextran 555 (for synaptic terminals), and Yoyo-1 (for nucleic acids) to study the presence and localization of peptides and proteins. We also used the selective perisynaptic schwann cell (PSC) ablation to specifically address the presence of proteins on the PSCs. NAAG, an acidic dipeptide, is the most prevalent peptide neurotransmitter in the mammalian central nervous system. We localized NAAG and its related proteins at the NMJ. NAAG co-localizes with synaptic vesicles and can be depleted from the nerve terminal using high potassium bathing solution. The NAAG receptor, metabotropic glutamate receptor type 3 (mGluR3) is present at the NMJ at both the presynaptic terminal and PSCs. mGluR2, known to bind glutamate, is located on the postsynaptic cell (i.e. the muscle). In addition to the receptor proteins, the hydrolytic enzyme glutamate carboxypeptidase II (GCPII) also is present on the PSCs. GCPII cleaves NAAG into N-acetylaspartate (NAA) and glutamate, thereby inactivating NAAG in the synaptic cleft. However, the byproduct glutamate can activate mGluR2 as well as NMDAR. The localization of GCPII to the PSCs was also confirmed using complement-mediated ablation of the PSCs. We implemented a novel reload study to determine where NAAG is packaged into synaptic vesicles using depletion and bath application of NAAG components. A putative NAA transporter is also present. Given the fact that NAAG is localized to synaptic vesicles and can be depleted and reloaded in the synapse and both the putative NAAG receptor and inactivating enzyme are present, we propose that NAAG functions as a neurotransmitter/neuromodulator at the lizard neuromuscular junction.
CARDIOVASCULAR, AUTONOMIC, AND THERMOREGULATORY EFFECTS OF CHRONIC EXERTIONAL HEAT STRESS IN RATS

Harald M. Stauss, Navita Choudhary, Abigail Nash, Frederick O. Liaboe, Kevin C. Kregel, Department of Health and Human Physiology, The University of Iowa, Iowa City IA.

In recent years, hospitalizations from heat-related injuries have been on the rise in the U.S. military and a growing concern as conflicts in the Middle East continue, leading to battlefronts in extremely hot and arid environments. In previous studies using a well-established rat model of exertional heat stress (ExHS), a single bout of exercise in the heat showed characteristic alterations in diurnal variations for both heart rate (HR) and systolic blood pressure (SBP). The current study was designed to investigate the cardiovascular, autonomic, and thermoregulatory effects of multiple bouts of ExHS. Rats were implanted with a telemetric probe that recorded blood pressure (BP) and core temperature ($T_c$) throughout the entire protocol. During the ExHS protocol, rats ($n=8$) were run on a motorized running wheel (7-11 m/min) at 39°C ambient temperature ($T_a$) until $T_c$ reached 41.8°C. Control rats ($n=7$) were placed in the wheel (0 m/min) at 23°C $T_a$ for 90 min (average ExHS protocol duration). The ExHS protocol lasted 5 days, and involved 10 ExHS bouts (twice a day with 8 h in between each protocol). Rats were housed in a telemetry room, with $T_c$ and BP recorded 3 days before, during, and 3 days after the 5-day ExHS protocol. Data analysis was done on a 1 h clean segment of BP data obtained between 12 am and 4 am, when there were minimal disturbances. Low frequency (LF) HR variability ($HR_{LF}$) and LF SBP variability ($SBP_{LF}$) increased from 13.4±3.4 bpm$^2$ and 6.0±1.0 mmHg$^2$ at baseline to 27.7±6.3 bpm$^2$ (P<0.05) and 13.8±2.0 mmHg$^2$ (P<0.05) in the night following the first protocol day and remained elevated throughout the nights following the five days of ExHS. Compared to baseline, HR and $T_c$ were temporarily elevated in the night following the first protocol day (368 ±11 vs. 317±4 bpm, P<0.05 and 38.8±0.04 vs. 37.7±0.08°C, P<0.05) but returned to baseline levels in the night following the fifth protocol day, suggesting a training and heat acclimation effect. High frequency spectral power of HR and time domain measures of HR variability showed no change in the nights following the ExHS protocol, suggesting no significant changes in cardiac parasympathetic modulation. In conclusion, the elevated nocturnal $HR_{LF}$ and $SBP_{LF}$ throughout the ExHS protocol, together with the blunted HR and $T_c$ responses at the end of the ExHS protocol, suggest that chronic exposure to ExHS can lead to a sustained increase in cardiac and vascular sympathetic activity despite training and heat acclimation.

Supported by funding from DoD/USAMRMC #W81XWH-10-2-0115.
The model plant Arabidopsis thaliana contains a family of twenty Glutamate-Like Receptors (GLRs). These genes are related to the glutamate-receptor ion channels found in the central nervous system and peripheral tissues of mammals. A major question since the discovery of these genes is what role they play in a plant, including their function and physiology. One of the members of this family, GLR3.4, was heterologously expressed in human embryonic kidney (HEK) cells and the ion channel function studied using whole-cell patch clamp. It was found that GLR3.4 conducts calcium across the plasma membrane of transfected HEK cells in response to specific extracellular amino acids. This function is very similar to the NMDA (n-methyl-d-aspartate) class of mammalian glutamate receptors, which are particularly involved in mediating the long-term potentiation (LTP), which is thought to be the mechanism underlying memory formation.
EFFECTS OF OMEGA-3 FATTY ACIDS ON 3T3-L1 PREADIPOCYTE DIFFERENTIATION AND INFLAMMATORY RESPONSE

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Obesity and in particular, adipose tissue (AT)-specific inflammation is associated with several metabolic disorders such as insulin resistance, dyslipidemia, and hepatic steatosis. Fish oil containing omega-3 fatty acids has been shown to reduce obesity in several animal models however this effect appears to be strain-specific. We and others have shown that fish oil can actually increase AT mass in animal models which is associated with reduced inflammation and hepatic steatosis. Therefore, the objective of this study is to determine whether the omega-3 fatty acids induce the differentiation process in 3T3-L1 preadipocytes which, in turn, leads to improved lipid storage in adipocytes. Methods and Results: 3T3-L1 cells were pretreated and differentiated in the presence of different long chain fatty acids such as oleic acid (OA), stearic acid (SA), eicosapentanoic acid (EPA), docosahexanoic acid (DHA), or a mixture of EPA and DHA (E+D) at a concentration of 50 BM. Lipid accumulation was analyzed by oil red O staining and the expression of different genes modulating differentiation and inflammatory response was analyzed by real time PCR. We noted that the differentiation process was remarkably increased in cells that were treated with omega-3 fatty acids compared to OA as analyzed by oil red O staining. Real time PCR analysis showed that the expression of aP2, a marker of preadipocyte differentiation, was increased significantly in cells treated with DHA (4.1-fold, \(P<0.001\)) or SA (2.8-fold, \(p<0.05\)). DHA and E+D but not SA also increased the expression of cEBP alpha, another marker of differentiation (8.0-fold and 6.2-fold in DHA and E+D, respectively, \(P<0.001\)). Importantly, the increased expression of differentiation markers upon treatment with omega-3 fatty acids is not associated with an increase in inflammatory genes, in particular, MCP-1. It is interesting to note that although SA treatment also led to a significant increase in aP2, this effect is associated with an increase in MCP-1 (1.9-fold, \(P<0.05\)). Conclusions: Our data demonstrate that both omega-3 fatty acids and SA can induce the differentiation of preadipocytes. Unlike SA, the differentiation induced by omega-3 fatty acids is not associated with an inflammatory response suggesting that this effect of omega-3 fatty acids is beneficial in improving the lipid storage function of AT without inducing an inflammatory response.
This study investigates the effects of virtual reality (VR) on human ratings of perceived exertion (RPE) and oxygen consumption (VO₂) during inclined treadmill walking in healthy young adults.

Participants walked on a treadmill at varying degrees of incline (grade of 0%, 3%, 6%) through a virtual corridor with varying virtual degrees of incline (manipulation of virtual environment at 0%, 3%, 6% grade). Oxygen consumption was collected using the Cosmed K4b2 system, and RPE was measured with Borg's scale of perceived exertion during each of the conditions.

Both RPE and oxygen consumption were significantly affected by an increased incline of the treadmill (p<0.05). Only the perception measure (RPE) was significantly influenced by an increased incline of the virtual corridor (p<0.05). This study confirms that the VR affects human perception of exercise intensity, but not the actual metabolic consumption during treadmill walking. It is possible that the increase in perceived exertion of exercise is due to the effect of visual input (VR) on the brain (CNS) and sensorimotor system.
FACTORS AFFECTING THE SUSCEPTIBILITY OF HIGH SCHOOL STUDENTS TO NOISE INDUCED HEARING LOSS DUE TO IPODS?

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The volume in which teenagers listen to their iPods is a health risk factor for noise-induced hearing loss. This study explores this statement by answering the question: What factors influence the susceptibility of students to noise-induced hearing loss due to iPods at Millard North High School? In order to model the student population at Millard North High School, a survey was conducted on a sample of 260 students. The survey was stratified by grades and four classes were randomly selected from each grade using a random number generator. The survey includes questions about how loud (in dB) teenagers are listening to their music, how long teenagers are listening to their music and what listening habits contribute to their volume levels. The survey will also ask for demographic information such as gender and age. In addition, a Sound Pressure Level Meter was used to measure the actual volume of the headphones that the students carry with them on the day of the survey. This survey does not test for actual hearing damage in the students. Neither does it cover populations outside Millard North High School. From the data gathered in this survey, there was no significant difference in the listening levels from grade to grade. The difference between listening levels of males and females was found to be statistically significant. (p=0.011) Students with lower GPAs tend to listen to music at a louder level. Students in non-honors classes also listened to music at a higher volume. (p=0.0085) However, the measurements of volume levels using the Sound Level Pressure Meter do not take into account the type of headphones used. Future studies should investigate the role that the type of headphones used have on the volume levels.
Graduate Student Posters
G1 – G22
THE IMMUNOPHENOTYPIC AND CYTOKINE CHARACTERIZATION OF THE 
OSTEOARTHRITIC HUMAN PATELLAR FAT PAD: COMPARISON TO SUBCUTANEOUS 
ADIPOSE TISSUE

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Adipose tissue is a metabolic and endocrine organ as well as a critical hematopoietic and immune cell depot. Subcutaneous and visceral adipose tissue inflammation characterized by increased lymphoid and myeloid cell numbers has been documented in human and murine obesity model studies. The hematopoietic cell infiltrates are thought to be contributory factors to diabetes and metabolic syndrome co-morbidities. Similar changes within the infrapatellar fat pad (IPFP) are implicated in knee osteoarthritis. The immunophenotypes of stromal vascular fraction and adipose-derived stem cells of the infrapatellar fat pad and subcutaneous adipose tissue were determined in tissues from osteoarthritic subjects (n =7) with a Kellgren Lawrence score of 3.4± 1.2 (mean ±S.D.) undergoing total knee replacement. Based on flow cytometry, cell populations in the infrapatellar fat pad resembled those within subcutaneous adipose tissue; with the exception of the endothelial marker CD31+ which was significantly greater in cells from subcutaneous tissue. Lower numbers of capillary-like structures and higher numbers of stromal and alkaline phosphatase colony forming units in the infra-patellar fat pad versus subcutaneous tissue support this finding. In conclusion, the infrapatellar fat pad contains an immune cell population similar to that of donor-matched subcutaneous adipose tissue. It remains to be determined if hematopoietic cell populations in adipose depots play an equivalent etiological role in osteoarthritis as they have been shown to play in diabetes associated with obesity.
BRAIN-SELECTIVE OVEREXPRESSION OF ACE2 ATTENUATES SYMPATHETIC NERVE ACTIVITY AND ENHANCES BAROREFLEX FUNCTION IN CHRONIC HEART FAILURE

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Angiotensin-converting enzyme 2 (ACE2) has been suggested to be involved in the central regulation of autonomic function. During chronic heart failure (CHF), elevated central angiotensin II (Ang II) signaling contributes to the sustained increase of sympathetic outflow. This is accompanied by a downregulation of ACE2 in the brain. We hypothesized that central overexpression of ACE2 decreases sympathetic outflow and enhances baroreflex function in CHF. Transgenic mice overexpressing human ACE2 selectively in the brain (SYN-hACE2) and wild type littermates (WT) were used. CHF was induced by permanent coronary artery ligation (CAL). Four weeks after CAL, both WT and SYN-hACE2 mice exhibited a significant decrease in left ventricular ejection fraction (<40%). A decrease in mean arterial pressure was found only in SYN-hACE2 mice (CHF: 90.8±4.4mmHg vs. Sham: 109.4 ± 3.7 mmHg; p<0.05). Compared with WT mice with CHF, brain-selective ACE2 overexpression attenuated left ventricular end-diastolic pressure (WT CHF: 15.3±2.7 mmHg vs. SYN-hACE2 CHF 7.4±2.2 mmHg; p<0.05); decreased urinary norepinephrine excretion (WT CHF: 623 ±72 ng/day vs. SYN-hACE2 CHF: 378±39 ng/day; p<0.05) and baseline RSNA (WT CHF: 71.6 ± 7.6 % Max vs. SYN-hACE2 CHF: 49.3 ± 6.1% Max; p<0.05). Chronic subcutaneous infusion of A779 increased RSNA in SYN-hACE2 mice with CHF (A779: 67.3 ± 5.8% vs. vehicle: 46.4 ± 3.6% of Max, p<0.05). Baroreflex sensitivity was enhanced (Maximum Slope: WT Sham: 1.61±0.16 vs. SYN-hACE2 CHF: 1.51±0.17%/mmHg, p<0.05). Finally, Western blot analysis showed that AT 1R expression was up-regulated in medullary 1 nuclei in WT CHF mice. This up-regulation was significantly attenuated in SYN-hACE2 mice with CHF. These data suggest that central ACE2 overexpression exerts a potential protective effect in CHF through attenuating sympathetic outflow. The mechanism for this effect involves Ang (1-7) mas signaling as well as a decrease in AT 1R in the medulla.
Dysregulation of angiotensin II (AngII)-dependent central neural mechanisms and the development of cardiovascular diseases, such as hypertension, are associated with increased superoxide in the brain. Adenoviral-mediated overexpression of the superoxide scavenging enzyme copper/zinc superoxide dismutase (CuZnSOD) in the brain inhibits angiotensin II (AngII)-induced hypertension. However, adenoviral gene therapy is limited by its toxicity and inability to target the brain with peripheral administration. To improve delivery of CuZnSOD to the brain, a nanotechnology delivery system was developed consisting of a polyion complex micelle with a polyethylene glycol (PEG) corona and a polyethyleneimine (PEI) core that electrostatically binds CuZnSOD protein to form so-called CuZnSOD nanozyme (CuZnSOD nano). Previously, we demonstrated that CuZnSOD nano abolishes the AngII-induced increase of superoxide in CATH.a neurons and that an intracarotid injection of CuZnSOD nano inhibits the pressor response induced by central AngII. In the current study, we hypothesized that CuZnSOD nano delivers CuZnSOD protein to neurons via an active endocytosis mechanism. Western blot analysis showed that CATH.a neurons express both clathrin and caveolin-1. Confocal microscopy revealed that these neurons internalize transferrin and cholera toxin subunit B (CTB), which utilize clathrin- and caveolae-mediated endocytosis, respectively. Using fluorescently-labeled CuZnSOD and cultured neurons, we observed a significant increase in the neuronal uptake of CuZnSOD nano (69.6 ± 2.4 arbitrary fluorescent units, AFU) versus free CuZnSOD protein (21.5 ± 0.96 AFU, P<0.05) following 6 h incubation. Cytochalasin D, a non-specific inhibitor of active endocytosis, blocked the internalization of CuZnSOD nano (15.2 ± 0.61 AFU, P<0.05 vs. CuZnSOD nano). In addition, chlorpromazine, an inhibitor of clathrin-mediated endocytosis, attenuated the uptake of CuZnSOD nano (13.6 ± 0.64 AFU, P<0.05 vs. CuZnSOD nano). Nystatin and methyl-beta-cyclodextrin, inhibitors of caveolae-mediated endocytosis, also decreased the uptake of CuZnSOD nano (28.0 ± 1.1 AFU and 28.6 ± 1.1 AFU, respectively, P<0.05 vs. CuZnSOD nano). Furthermore, CuZnSOD nano co-localizes with transferrin and cholera toxin subunit B near the plasma membrane. These data collectively indicate that CuZnSOD nano is internalized through a combination of clathrin and caveolae-dependent endocytosis. Co-localization studies with fluorescent organelle markers show that CuZnSOD nano localizes partially in lysosomes (38.5%), Rab5+ endosomes (17.1%), mitochondria (9.8%), tubulin (24.0%), and endoplasmic reticulum (5.2%). Together, these data identify PEI-PEG polymer as a delivery system for potentially therapeutic antioxidant proteins, such as CuZnSOD, which are otherwise membrane impermeable.
CHI-SQUARE ANALYSIS OF MENDELIAN INHERITANCE OF FOUR GENES IN AN UNDERGRADUATE GENETICS LABORATORY EXPERIMENT

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We have developed an accessible protocol for using zebrafish to teach Mendelian genetics, scientific method, and statistical analysis in undergraduate laboratory courses. In these experiments, students gather raw data, generate hypotheses about Mendelian inheritance patterns, and test their hypotheses using chi-square statistical analysis. Zebrafish carrying mutations and transgenes are used to challenge students' understanding of Mendelian genetics. In the simplest case, a cross between two adults heterozygous for the cyclops mutation is used to demonstrate inheritance of a recessive allele. Approximately one quarter of the resulting progeny are cyclops homozygotes, and have cyclopic eyes, while approximately three quarters have a wildtype phenotype. The squint mutant is used to learn about incompletely penetrant phenotypes, as only a fraction of fish homozygous for squint express the mutant phenotype. Commercially available GloFish® contain Glo transgenes, which use a muscle specific promoter to drive expression of Green Fluorescent Protein, Red Fluorescent Protein (RFP), or Yellow Fluorescent Protein (YFP). In the most complex experiment, students analyze a trihybrid cross between fish carrying GloYFP, GloRFP, and golden (which causes fish to lose their stripes). GloYFP and GloRFP are incompletely dominant with each other. Alone, they make the fish yellow or red. Combined they produce an orange fish. This trihybrid cross provides complex calculations for students to perform during the chi-square analysis. Students also gain practice in analyzing complicated phenotypes, such as traits that are controlled by more than one gene. Zebrafish are an excellent genetic model organism for the classroom because of attributes such as easily scoreable mutants, large clutch sizes, low facility maintenance, and excellent availability. This set of experimental protocols, the raw data, and the analyses have been made widely available in the main text and supplemental materials of our manuscript (Zebrafish 2011, 8: 41-55.). The data include the numbers of progeny with specific phenotypes, pictures of each fish, and the chi-square analyses, enabling students without access to zebrafish to conduct virtual experiments.
A characteristic of chronic heart failure (HF) that increases the risk of mortality is elevated sympathetic drive. The paraventricular nucleus (PVN) of the hypothalamus is involved in the neural control of sympathetic drive. The activation of the PVN may be influenced by input from higher forebrain areas such as the subfornical organ (SFO). We hypothesized that an activated SFO contributes to the enhanced sympathetic activity during HF. Sprague Dawley rats were subjected to coronary artery ligation to induce HF. HF was confirmed by echocardiography and left ventricular pressure measurement four weeks after surgery. The activation of the SFO was analyzed by immunohistochemistry FosB staining and western blotting. Rats with HF displayed an increase in FosB-positive cells in the SFO compared to sham rats (29 vs. 101 Fos-B positive cells). HF rats also exhibited an elevated protein expression of angiotensin II (Ang II) type 1 receptor (0.21 vs. 0.35 AU) and endothelin receptor B (3.07 vs. 3.28 AU), and a decreased level of GABA\textsubscript{A} receptor (1.04 vs. 0.6 AU). Moreover, in urethane-anesthetized rats, A microinjection of 200 pmol of Ang II into the SFO increased renal sympathetic nerve activity, blood pressure, and heart rate to a greater extent in HF than in sham rats (change in RSNA: 14.6 vs. 31.1% basal value). In conclusion, the enhanced activation of SFO may contribute to the increased sympathoexcitation in HF.
PGC1 ALPHA OVER-EXPRESSION RESCUES DYSTROPHIN DEFICIENT SKELETAL MUSCLE

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Utrophin replacement has shown great promise as a therapy for DMD, however, a practical means of delivery in human patients is lacking. PGC-1alpha has been shown to increase utrophin and expression of oxidative proteins, which may be of additional benefit to dystrophic muscle. Previously, transgenic PGC-1alpha over-expression prevented the typical phenotypic decline in mdx mice. Further, we have shown that neonatal PGC-1alpha induction blunted acute eccentric injury in mdx mice. As boys are generally diagnosed following disease onset it is imperative to determine the extent to which PGC-1alpha over-expression can rescue skeletal muscle with active disease pathology. Three week old mdx mice were injected in the left hind limb with null AAV6 and in the right hind limb with an AAV6 driving expression of PGC-1alpha (1x10^11 gc). At six weeks of age, mice were euthanized and muscles removed. PGC-1alpha over-expression reduced total damaged area by 37% (p<0.05). More specifically, the areas of hypercontracted cells, immune cell infiltration, and missing cells were reduced by 65%, 31%, and 43%, respectively (p<0.05). Also utrophin protein expression was increased nearly 3-fold (p<0.05) in the PGC-1alpha treated limb. These data indicate that PGC-1alpha gene transfer following postnatal development of the disease can confer a high degree of improvement to dystrophic muscle. This investigation justifies further exploration of PGC-1alpha as a potential avenue for treating patients with DMD. Partially supported by CIAG.
NEURONAL UPTAKE OF CROSS-LINKED COPPER/ZINC SUPEROXIDE DISMUTASE

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Excessive production of reactive oxygen species (ROS), such as superoxide, in the central nervous system has been implicated in many brain-related pathological conditions including hypertension and heart failure. However, the therapeutic potential of antioxidant therapies has been limited by restricted transport across the blood-brain barrier (BBB) and plasma membrane, poor pharmacokinetics, and insufficient bioavailability. To address these limitations, we developed a stable nanoformulated antioxidant polyion complex in which copper/zinc superoxide dismutase (CuZnSOD or SOD1) protein is wrapped with cationic block copolymers that are covalently cross-linked. Herein, we tested the hypothesis that this cross-linked SOD nanozyme is more efficient in delivering SOD protein to central neurons than free SOD protein without inducing significant cellular toxicity. Central neurons from mice (CATH.a neurons) were exposed to cross-linked SOD nanozyme or free SOD protein for 1, 3, 6, or 24 hours, and cell viability and cellular uptake of SOD were assessed. One-hour exposure with cross-linked SOD nanozyme modestly, but significantly reduced cell survival versus vehicle-treated neurons as measured immediately following removal of nanozyme from the neuronal culture (89 ± 2% survival, P<0.05); this reduced cell survival was also observed after 24 hours (91 ± 3% survival, P<0.05) and 48 hours (88 ± 3% survival, P<0.05). However, treatment with free SOD protein for 1 hour had no significant affect on cell survival at these time points when compared to vehicle-treated neurons. In addition to the viability studies, confocal microscopy was used to investigate the neuronal uptake of cross-linked SOD nanozyme versus free SOD protein by utilizing fluorescently labeled cross-linked SOD nanozyme or free SOD protein. Robust cellular uptake of the cross-linked SOD nanozyme was observed within 1 hour of exposure, whereas cellular uptake of the free SOD protein was not apparent until after 3 hours of exposure. These data suggest that there is increased neuronal cell uptake of cross-linked SOD nanozyme following exposure as compared to free SOD protein, while only reducing cell survival minimally (i.e. ≤ 15%). Further experiments need be conducted to assess the mechanism(s) of cross-linked SOD nanozyme uptake and bioavailability, polymer biodegradation and subsequent effects on cell viability.
Role of CFTR in IC-Beta Cells in Na-Independent K Secretion

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Classically, the mechanism of K secretion involves exchange for Na reabsorption in the distal nephron. However, ancient man consumed a low Na, high K diet and the Yamamoto Indians of South America still consume a high K (HK) diet that is nominally free of Na ions. We have previously shown with BK-beta4 knockout mice that the BK-beta4 channel of the distal nephron intercalated cells (IC) has an important role in Na-independent K secretion. Also, the transtubular K gradient (TTKG) across the distal nephron indicates a large lumen negative potential that is independent of Na reabsorption. We hypothesized that for animals on a HK diet that K is secreted independently of Na reabsorption by a mechanism in IC-beta cells involving HCO3 secretion via pendrin-mediated HCO3/Cl exchange and Cl recycling across the apical membrane via the cystic fibrosis transmembrane conductance regulator (CFTR). In support of this hypothesis, we found that urinary pH of mice on a HK diet was 8.3, whereas urinary pH of mice on a control diet was 6.0. The proportion of IC-beta, compared with IC-alpha increased from 30% in mice on a control diet to 60% in mice on a HK diet. Moreover, IC-beta cells from HK diet mice exhibited significantly greater (70% increase) staining intensity of H-ATPase compared to control diet. In control, and HK dietary conditions, CFTR was detected on the apical membrane in a portion of IC cells by immunohistochemical staining. Double staining with H-ATPase and CFTR revealed the localization of CFTR on the apical membrane of only IC-beta cells. Also, CFTR staining increased in mice on a HK diet compared to control diet. These data indicate that for HK diet mice that (1) HCO3 secretion via IC-beta is enhanced and (2) CFTR expression is localized in the apical membrane of IC-beta where it is necessary to recycle luminal Cl thereby serving as a Na-independent driving force for K secretion.
Primordial follicle formation is the first step in ovarian follicular development in mammals. The initial pool size of primordial follicles determines the lifetime quota of available oocytes and thereby fertility. Defects in primordial follicle formation may lead to premature ovarian failure (POF). Estrogen (E) has been shown to affect this process, but the mechanism is unknown. E is known to activate ERBB3-mediated signaling, a known mitogenic pathway. ERBB3 is associated with a repressor protein EBP1, which dissociates upon phosphorylation resulting in ERBB3 activation. We hypothesize that E promotes primordial follicle formation by regulating the expression and/or function of ERBB3 and its repressor EBP1. The objective of this study was to determine whether the expressions of ERBB3 as well as its repressor, EBP1, are regulated by E in ovarian cells during somatic cell and oocyte assembly forming primordial follicles. We used perinatal hamster ovaries for this study. Western blot and immunofluorescence of ovaries of treated and untreated hamsters were used to investigate our questions. The Western blot analyses showed that EBP1 expression was downregulated in 8-day old (P8) ovaries containing primordial follicles compared to 15-day old fetal (E15) ovaries lacking any follicle (0.73 ± 0.171 vs. 1.322 ± 0.359 P < 0.05). ERBB3 expression on the other hand was upregulated. Whereas pEBP1 (ser 363) was localized primarily in the oocytes of E15 ovaries, it was mostly localized in somatic cells juxtaposed to the oocytes and also in the granulosa cells of P8 ovaries. E treatment on P8 suppressed EBP1 expression at 4hr and 24hr (0.229 ± 0.130) time points but a 7-day long treatment, (injections on P1 and P4) did not significantly alter EBP1 levels on P8 (0.807 ± 0.194) compared to control, suggesting a possible rebound of EBP1 expression following its initial suppression. Twelve-day old fetuses (E12) were treated in utero with an FSH-antiserum (FSH-AS) to reduce the effective levels of serum FSH, and consequently of E levels in P8 hamsters. FSH-AS significantly upregulated EBP1 expression compared to P8 (P < 0.01) but E replacement on P1 or P4 did not have a significant lowering effect. In contrast, ovarian ERBB3 expression was reduced on P8 in the antiserum treated group compared to untreated animals. E injection on P1 or on P1 and P4 significantly upregulated ERBB3 expression compared to antiserum treated animals. These results suggest that EBP1 and ERBB3 protein expression is inversely related in postnatal hamster ovaries, especially during primordial follicle formation, and their expressions are regulated by E. Because ERBB3 activation is facilitated by EBP1 downregulation, it is logical to speculate that ERBB3 activation by E may play a critical role in primordial follicle formation.
INCREASED METHYLGLYOXAL IS AN UNDERLYING CAUSE FOR CEREBRAL VASCULAR LEAKAGE IN BRAIN OF TYPE 1 DIABETIC RATS

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Learning and memory deficits are increasingly being recognized in children with type 1 diabetes. Data from several laboratories including ours, suggest these defects result from diabetes-induced damage to the cerebral vasculature. To date, mechanisms responsible for this damage remain unknown. This study was designed to determine if the cytotoxic α-oxoaldehyde methylglyoxal (MGO) generated during diabetes is an underlying cause. After 8 weeks of streptozotocin-induced type 1 diabetes, MGO levels increased 200-fold in rat brain (from 1.0 μM/200mg tissue to 200 μM/200 mg tissue). Intravenous infusion of fluorescein isothiocyanate (FITC)-labeled albumin into diabetic rats showed vascular leakage in cortex, hippocampus and cerebellum. These regions also contained increased amounts of TUNEL-positive cells. Western blot also revealed elevated levels of C/EBP homologous protein (CHOP), the endoplasmic reticulum (ER) stress apoptotic marker, in the cortex. Magnetic resonance imaging also showed leakage in cortex, hippocampus and cerebellum of control rats administered MGO for 21 days. Increasing expression of glyoxalase-1, the enzyme responsible for degrading MGO, prevented cerebral vascular leakage in type 1 diabetic rats. These data implicates MGO as an underlying cause for cerebral vascular leakage and likely neuronal dysregulation/death during type 1 diabetes.
SYNTHESIS AND CHARACTERIZATION OF A RYANODINE RECEPTOR 1-SELECTIVE AGONIST

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Ryanodine receptor (RyR) are Ca\(^{2+}\) release channels on the sarco(endo)plasmic reticulum (SR/ER) through which Ca\(^{2+}\) leave the ER/SR to initiate a range of important physiologic functions including skeletal muscle and cardiac contractions, neurotransmitter release, gene transcription and synaptic plasticity (learning and memory). Three isoforms of RyR are present in mammals, RyR1 found in skeletal muscle, RyR2 which is the major isoform in the heart and RyR3 which is present in diaphragm as well as in the brain. To date, few ligands exist that can discriminate between RyR isoforms. The present study describes synthesis and characterization of 2-cyclohexyl-3-cyclohexylimino-2,3-dihydro-pyrrolo[1,2-c]-imidol-1-one, a small molecule that selectively activates RyR1. This ryanodine receptor 1 selective agonist (RSA1) was synthesized by reacting pyrrole-2-carboxylic acid with N,N”-dicyclohexylcarbodiimi de in the presence of dimethyl amino pyridine in anhydrous dichloromethane under a vacuum and its chemical structure was confirmed using \(^1\)H, \(^13\)C and X-ray crystallography. In binding affinity assays, RSA1 enhanced the binding of \(^{3}\)H\)ryanodine to RyR1 in a concentration-dependent manner (250% over control with 50\(\mu\)M) with minimal effects on RyR2 and RyR3. The ability of RSA1 to increase \(^{3}\)H\)ryanodine binding was calcium-dependent but independent of reduced glutathione. In lipid bilayer assays RSA1 increased the open probability of RyR1 with no effect on the open probability of RyR2 or RyR3. RSA-1 also increased cytoplasmic Ca\(^{2+}\) in skeletal muscle C\(_2\)C\(_{12}\) cells that was independent of external Ca\(^{2+}\). These data identifies RSA-1 as a novel RyR1-selective agonist.
Clinical studies suggest enhanced blood pressure variability (BPV) may lead to cardiovascular damage even in normotensive subjects. The purpose of this study was to investigate the time course of cardiovascular responses to enhanced BPV secondary to a partial loss of baroreflex function. Male Sprague Dawley rats were instrumented with a telemetric blood pressure sensor and were randomly assigned to either the sinoaortic denervation group (SAD) or the sham surgery, control group (Sham). During an observation period of six weeks, structural and functional cardiac (echocardiography) and microvascular (iris imaging of the long posterior ciliary artery, LPCA) parameters were assessed weekly. After the observation period, left ventricular end diastolic pressure (LV EDP) and cardiac contractility (dP/dt_{max}) were determined and the heart, aorta, and carotid arteries were collected for further analyses. SAD resulted in enhanced BPV and a decreased baroreflex gain (-32%) without increasing mean blood pressure. In vivo imaging of the LPCA in SAD demonstrated decreased endothelial-mediated dilation in response to corneal application of pilocarpine but not to the exogenous nitric oxide donor sodium nitroprusside. SAD also demonstrated a blunted dilatory response to prostacyclin. Histology of the aorta and carotid artery suggested an increased wall thickness (+18%) and wall/lumen ratio (+16%) following SAD. Furthermore, aortic distensibility measured by echocardiography was decreased (-54%) in SAD compared to Sham rats. Despite final heart weight/body weight ratio being similar in both groups, SAD tended to have elevated LV EDP (+8%) and a loss of cardiac contractility (-10%). In summary, exposure to enhanced BPV for a six week period resulted in structural and functional changes in both, small resistance and large conduit arteries. Endothelial dependent dysfunction may involve changes to both nitric oxide and prostacyclin pathways. Additionally, early cardiac systolic and diastolic dysfunction was evident, suggesting cardiac fibrosis in SAD. The results of this study demonstrate that BPV constitutes an important cardiovascular risk factor that is independent of the mean level of arterial blood pressure.
CHANGES IN RENAL BLOOD FLOW DURING INFUSION OF PHENYLEPHRINE AND NOREPINEPHRINE IN RATS WITH CHRONIC HEART FAILURE

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Chronic heart failure (CHF) is a disease characterized by an overactive sympathetic nervous system. The disease, although originating in the heart, eventually affects the function of multiple organs and their control mechanisms. One target of this disease, the kidney, is especially vulnerable to these adaptations, and responds to the increased renal sympathetic nerve activity (RSNA) with a decrease in renal blood flow (RBF) which can contribute to a progressive decline in renal function. The purpose of this study was to determine the effects of the $\alpha_1$ adrenergic receptor agonist phenylephrine (PE) and the nonselective adrenergic receptor agonist norepinephrine (NE) on RBF in sham and CHF animals. Four weeks prior to experimentation, animals were infarcted by coronary artery ligation. Cardiac function was assessed by echocardiography. Animals underwent an anesthetized preparation in which a flow probe was placed around the renal artery and a catheter in the aorta close to the renal bifurcations. Changes in renal blood flow were measured in response to intra-aortic infusions of increasing doses of PE (5, 10, and 20 $\mu$g/kg) and NE (8 and 16 $\mu$g/kg). Renal cortices from CHF and sham animals were sieved to obtain a vascular-enriched preparation. Sieved lysates were subjected to western blotting under standard conditions. CHF animals exhibited a blunted change in RBF in response to intra-aortic infusion of PE compared to sham animals at all concentrations. Conversely, CHF animals exhibited an increased sensitivity to NE. Given the differing responses to adrenergic receptor stimulation in CHF animals, we then performed western blotting on sieved kidneys to determine if the changes in blood flow in response to ligand correlated to differential expression of the targeted receptors. Preliminary western blotting data indicate that relative amounts of $\beta_1$ adrenergic receptor were significantly decreased in CHF animals compared to sham. Results of this study suggest that changes in RBF during CHF are due in part to alterations in adrenergic receptor signaling. Future experiments will examine the molecular mechanisms underlying these results.
ANGIOTENSIN II REGULATES ACTIVITY OF KINASES AND PHOSPHATASES IN NEURONS

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Angiotensin II (AngII), an important signaling molecule of the renin-angiotensin system in the central nervous system, plays a critical role in the regulation of systemic cardiovascular function. Upon acting on AT1 receptors AngII increases neuronal firing rate by elevating levels of reactive oxygen species (ROS), which in turn modulate ion channel activity. Previous studies have demonstrated that ion channel activity is also mediated, at least in part, by cellular kinases, such as calcium/calmodulin kinase II (CaMKII) and protein kinase C (PKC), and phosphatases, like protein phosphatase 2A (PP2A). However, the potential cross talk between AngII signaling and these redox sensitive proteins in neurons remains unclear. Here, we tested the hypothesis that AngII stimulation of central neurons influences the protein level and activity of CaMKII, PKC δ and PP2A. Mouse CATH.a neurons were treated with AngII (100nm) for various time points and protein levels of PP2A, CaMKII, and PKC δ were measured by Western blot analysis. Activity of CaMKII was assessed by detecting its phosphorylated levels; while PP2A activity was determined using a ser/thr Malachite Green phosphatase activity assay. AngII significantly increased levels of phosphorylated CaMKII from 2 hr (3.17-fold increase vs. vehicle stimulation, P<0.05) to 24 hr (3.19-fold increase vs. vehicle, P<0.05) of stimulation, thus indicating that AngII increases CaMKII activity. In addition, AngII significantly elevated PKC δ protein levels after 24 hr (2.63-fold increase vs. vehicle, P<0.05) of stimulation. In contrast, AngII modestly, but not significantly, decreased protein levels of PP2A from 30 mins up to 24 hrs of AngII stimulation versus vehicle stimulation. AngII also decreased PP2A activity within 10 mins of stimulation and up to 1 hr (818 µg of phosphate released at 10 mins and 794 µg at 1 hr vs. 1666 µg of phosphate released in vehicle-stimulated cells). Together, these data indicate that in neurons AngII inhibits activity of PP2A while activating CaMKII.
EXPRESSON OF ESTROGEN RECEPTOR ALPHA 36 IN THE HAMSTER OVARY: POSSIBLE REGULATION BY GONADOTROPINS AND STEROID HORMONES.

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Estradiol-17b (E), acting via its cognate receptors affects ovarian functions in mammalian females. Estrogen receptor alpha 36 (ERalpha36) is a splice variant of classic ESR1 and has role in non-genomic E signaling in cancer cells. The objectives of the present study were to examine by immunoblotting and immunofluorescence localization, whether ERalpha36 was expressed in the hamster ovary throughout the estrous cycle, and whether the expression was regulated by follicle stimulating hormone (FSH), luteinizing hormone (LH), E and progesterone (P). Immunoblot data indicated that ERalpha36 expression declined by proestrus (D4:0900 h compared to earlier days of the estrous cycle (D3:0900 h, 5.9 ± 1.4 vs. D4:0900 h, 2.7 ± 0.3; p<0.05) and remained low until D4:1600 h. Immunofluorescence results corroborated the findings and revealed that ERalpha36 was expressed only in the cell membrane of both follicular and interstitial cells. Hypophysectomy resulted in a significant decline in ERalpha36 protein levels (Hx: 3.4 ± 0.3 vs. D1: 10 ± 2.8; p<0.01). ERalpha36 protein expression in FSH-treated (8.8 ± 0.8) or LH-treated (8.8 ± 0.6) hypophysectomized (Hx) hamsters were upregulated (p<0.05), which is comparable to those of Day 1 hamsters (p>0.05), and also increased in Hx hamsters treated with combined dosage of FSH and LH (6. 4 ± 0.4). Neither E nor P alone or combined could affect ovarian ER alpha36 levels. The data were consistent with immunofluorescence findings. These results indicate that the ER alpha36 is translated into protein in ovarian follicular and non-follicular cells, and is localized in the cell membrane. Further, the induction of alternate ESR1 mRNA splicing and the translation of the truncated transcript seems to be regulated by FSH as well as LH.

(Values are expressed in Mean OD ± SEM).

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It has been suggested that uremia in patients with end-stage renal disease (ESRD) is strongly associated with autonomic neuropathy, leading to reduced heart rate variability (HRV) that is associated with increased cardiovascular morbidity and mortality. In addition, patients with ESRD are at a higher risk for endothelial dysfunction. Both, autonomic neuropathy and endothelial dysfunction contribute to the greater incidence of cardiovascular events in patients with ESRD. The hypothesis of our study was that renal transplantation reduces cardiovascular risk in patients with ESRD partly by improving endothelial and autonomic dysfunction.

To test this hypothesis, we studied endothelial vascular function and cardiac autonomic control in patients with ESRD before and 3 months, 12 months, and 36 months following renal transplantation (RT). Vascular function was studied by flow mediated dilatation (FMD) of the brachial artery and cardiac autonomic function was assessed by time- and frequency-domain HRV analysis.

Compared to before RT, FMD increased by 50% at one year after RT, indicating improved endothelial vascular function. During the first year following RT, time- and frequency-domain HRV parameters tended to increase (e.g., RMSSD 20.7±1.9 ms before RT vs. 25.3±5.4 ms one year after RT), indicating improved autonomic cardiac control and suggesting reduced cardiovascular risk. Compared to one year following RT, HRV parameters tended to be slightly lower at 3 years following RT (e.g., RMSSD 21.7±2.3 ms), but were still greater than before RT. Importantly, HRV (SDNN) correlated significantly (R=0.4, P<0.05) with FMD, suggesting that improved endothelial vascular function in response to RT is associated with improved HRV and, therefore, with reduced cardiovascular risk.

We conclude that RT in ESRD patients chronically improves endothelial vascular function and cardiac autonomic control for at least 3 years following RT. Because high HRV is associated with reduced cardiovascular risk and because improved endothelial function following RT was correlated with increased HRV, we speculate that the combination of improved endothelial vascular function and improved cardiac autonomic control contributes to reduced cardiovascular events following renal transplantation in ESRD patients.

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BK EXPRESSION IN HEK293 AND EFFECTS OF THE NMDA ACTIVATOR UBP684

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Large Ca\(^{2+}\)-activated K\(^+\) channels (BK) are important for regulating membrane potential in excitable tissues such as the brain and vasculature, and for secreting K\(^+\) in the kidney. NMDA receptors have previously been shown to be associated with BK, and their activation increases BK activity, likely due to calcium influx through the NMDA receptor. However, it was not established whether BK were directly activated by NMDA activators. We therefore hypothesized that the NMDA receptor activator, UBP684, would cause an increase in BK activity in the absence of NMDA. HEK293 cells transfected with the alpha subunit of BK (HEK293-BK) were placed in a physiological bath solution containing 150mM NaCl, 5mM KCl, 1mM CaCl\(_2\), 2mM MgCl\(_2\), 5mM Glucose, and 10mM HEPES and patched with pipettes containing either 140 mM KCl and 10mM HEPES (control) solution or 140 mM KCl, 10mM HEPES, and 100\(\mu\)M UBP684 (UBP684) solution. Polished pipettes with a resistance between 7-14 megohms were used for patching. BK channel density averaged 1.9 ± 0.5 (Mean ± SEM) channels per patch. Cell-attached patch configuration conductance was observed at 154 pS, and inside-out patch configuration conductance (inward currents) was observed at 198 pS. On depolarization (cell-attached; -V\(_p\) = 100mV) there was no significant difference (P=0.174) in BK activity between the control value of 0.051 ± 0.023 (N\(_{Po}\) ± SEM) and the UBP684 value of 0.052 +/- 0.017 (N\(_{Po}\) ± SEM). We conclude that: (1) the channel density and conductance values support use of HEK293-BK cells as an appropriate expression system for BK studies, and (2) our results reject the hypotheses that UBP684 directly activates BK.
EFFECTS OF MENOPAUSE ON KISSPEPTIN (KP) EXPRESSING NEURONS IN THE MBH AND POA IN FEMALE RHESUS MACAQUES

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Kisspeptin is a neuropeptide that regulates the release of gonadotropin releasing hormone (GnRH) in the hypothalamus. This project investigates changes in KP neurons in the medial basal hypothalamus (MBH) and preoptic area (POA) of postmenopausal macaques. Brain tissues from postmenopausal and eugonadal monkeys were cut at 50μm thickness. Serial sections at 100μ intervals were immunostained with GQ2, a KP antibody. Results show that KP somata were found in the infundibular nucleus and periventricular regions encompassing medial to the infundibular nucleus through medial to the paraventricular nucleus. The KP neurons in the MBH of the postmenopausal monkeys are larger in size than eugonadal monkeys. These results suggest that in the menopausal state, KP expression increases because of a decrease in the negative feedback of ovarian steroid hormones.
Previous studies at the University of Northern Iowa have indicated a potentially unique blood clotting inhibitor in hibernating Wood turtles (Glyptemys insculpta). The purpose of this study is to determine if similar anticoagulation mechanisms exists in other poikilotherms during hibernation. Twenty American Bullfrogs (Rana catesbeiana) were placed in a cold room, where hibernation at 3°C was gradually induced. Blood samples were taken during three different stages: pre-hibernation, hibernation, and post hibernation and the resulting plasma stored at -70°C. Tests for APTT clotting time and tests for the determination of the presence of an inhibitor were performed on the plasma of all frogs using a KC 1 Amelung blood coagulation analyzer. APTT times for plasma from hibernating frogs were greater than 1,000 seconds (no clotting), while non-hibernating frogs had an average APTT time of 190 seconds. Tests for the presence of a clotting inhibitor in the plasma of hibernating frogs found no evidence for such an inhibitor. Based on preliminary results, the prolonged APTT time in hibernating frogs is likely due to the decrease or absence of one or more clotting factors of the intrinsic coagulation pathway during periods of hibernation. These results differ from those of the Wood turtle suggesting that various mechanisms may exist in poikilotherms to reduce blood coagulation under the conditions of hibernation.
ATTENUATED PARASYMPATHETIC NERVOUS SYSTEM CONTROL OF THE HEART IN THE AGING MOUSE

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The autonomic nervous system maintains homeostasis through the complex balance of the sympathetic nervous system (SNS) and parasympathetic nervous system (PSNS). Especially evident in the heart, where parasympathetic activation affects sinoatrial nodal pacemaker activity and atrioventricular nodal conduction via the vagal nerve, maintenance of this balance is important for control of heart rate, conduction, and contractility. It is known that aging and heart disease both result in an increase in SNS activity and a decrease in PSNS activity, which may contribute to age-related cardiac dysfunction and remodeling. However, the mechanisms underlying this autonomic dysfunction, particularly impairment of PSNS function in aging, remains to be fully understood. Intracardiac ganglia relay and integrate the PSNS signals to the heart. This study investigated the major cholinergic components of intracardiac ganglia in the aging state. Utilizing young (1-2 month) and old (11-12 month) mice, this study shows that aging mice exhibit diminishment of baroreflex sensitivity and decline of response to rostral-severed cardiac vagal stimulation. However, this aging model exhibits maintenance of muscarinic acetylcholine receptor (mAChR) function comparable to young animals, as evidenced by preserved response to administration of mAChR agonist, Bethanechol. Analysis of protein expression of aging mouse atria reveals a decrease in acetylcholine synthesizing protein, choline acetyltransferase (ChAT), as well as a decrease in acetylcholine packaging protein, vesicular acetylcholine transporter (VAchT). In contrast, an upregulation of the rate-limiting protein, choline transporter (CHT), is seen in the aging animals and specifically in the cytosolic fraction. Additionally, immunohistochemistry of cardiac atria further suggests that these changes occur within intracardiac ganglia. These findings point towards altered cholinergic signals in the intracardiac ganglia as being involved in the attenuation of PSNS control of the heart in the aging mouse.
EXERCISE TRAINING REDUCES THE OCCURRENCE OF CARDIAC ARRHYTHMIAS AND CHANGES THE PATTERN OF LEFT VENTRICULAR CONNEXIN 43 EXPRESSION IN AGED RATS

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The occurrence of cardiac arrhythmias increases with age, although the mechanisms responsible for this effect are poorly understood. Exercise training (ET) has been shown to reduce the incidence of cardiac arrhythmias likely through improved cardiac sympathovagal balance. We hypothesized that aging would result in increased arrhythmogenesis at rest and during an acute stressor, while ET would prevent these effects in young (4-6 mo) and aged (24-25 mo) F344 rats. Rats underwent 10-11 weeks of treadmill training (11-14 m/min, 60 min/day, 5 day/week) or equivalent sedentary handling protocol. Subcutaneous electrocardiographic (ECG) leads were implanted aseptically at the end of the young sedentary (YS, n=6), young exercise (YEx, n=6), aged sedentary (AS, n=6), and aged exercise (AEx, n=6) training protocols to allow for ECG data acquisition via the Actiwave telemetry system. The arrhythmic index was calculated using a modified scoring system at baseline (BL), followed by an acute stressor consisting of sympathetic stimulation (isoproterenol (ISO), 0.15mg/kg, s.c.), and brief restraint (BR). Data indicate that physiological adaptations were consistent with ET in both young and aged rats as evidenced by an increase in the left ventricular to body weight ratio and a decrease in resting heart rate. Furthermore there was an increase in citrate synthase activity from the soleus muscle in YEx rats and a trend toward increase in AEx versus sedentary counterparts. The total arrhythmic index (BL+ISO+BR) was significantly higher in aged verses young rats while ET showed a trend toward reversing this effect in aged rats only. Connexin 43 (Cx43), the predominant myocardial gap junction protein, is essential for normal ventricular rhythmicity. Reduced left ventricular expression and change in phosphorylation status of Cx43 is associated with increased arrhythmogenesis. Therefore, we were also interested in the expression and phosphorylation status of Cx43 under exercise conditions of young and aged rats. Interestingly, YS and YEx rats have similar Cx43 expression patterns and no difference in arrhythmogenesis. In contrast, AS and AEx rats differ in their Cx43 expression patterns as well as well as having increased arrhythmogenesis. These preliminary data support the hypothesis that ET provides a protective benefit against sympathetically induced arrhythmogenesis in aged rats. Future studies will further investigate the role of Cx43 in mediating the increased arrhythmogenesis observed with aging and changes associated with ET.
THE ANTIOXIDANT METHIONINE SULFOXIDE REDUCTASE-A PROTECTS AGAINST ANGIOTENSIN-INDUCED CARDIAC DYSFUNCTION AND AORTIC ANEURYSMS INDEPENDENT OF CHANGES IN WHEEL-RUNNING ACTIVITY


Methionine sulfoxide reductase A (MsrA) reverses oxidation of methionine residues in proteins and protects tissues from oxidative injury. Previous studies have shown that MsrA deficient mice exhibit increased sensitivity to oxidative stress, decreased locomotor activity, and reduced lifespan. We hypothesized that chronic infusion of angiotensin II (Ang-II), a hormone known to cause oxidative stress and hypertension, would cause left ventricular (LV) dysfunction, arterial damage, and reduced exercise capacity in young 10-16 week old MsrA-/-mice; while control C57BL6 mice would be resistant to these effects. Four groups of mice were studied: MsrA-/-mice infused with either Ang-II (1000 ng/kg/min, n=8) or saline (n=7) for 28 days (osmotic minipump), and control mice infused with either Ang-II (n=7) or saline (n=8). Voluntary wheel-running activity was measured daily. LV function and ascending aortic diameter were assessed by echocardiography under baseline conditions and after 4 weeks of Ang-II or saline infusion. Mice were euthanized, and histopathological analyses were performed on the heart and arterial tree. Measurements of LV function and ascending aortic diameter were similar in saline-infused control mice, Ang II-infused control mice, and saline-infused MsrA-/-mice. In contrast, Ang II-infused MsrA-/-mice exhibited decreased LV ejection fraction, increased LV volume/mass ratio, and increased ascending aortic diameter (P<0.05). Furthermore, aortic aneurysms were observed only in Ang II-infused MsrA-/-mice. The effect of Ang-II on wheel-running was variable in both genotypes and did not predict aortic dilatation or aneurysm. We conclude: (1) MsrA protects against Ang II-induced cardiac dysfunction and development of aortic aneurysms, presumably by reducing oxidative damage; and (2) the protection is not associated with improved exercise capacity as measured by wheel-running activity.
Post-doctoral Research Associate Posters P1 – P18
Using a type-1 diabetic rat model, our lab has previously shown that elevation of endogenous angiotensin II (Ang II) in nodose ganglia (NG) is associated with enhanced currents of hyperpolarization-activated cyclic nucleotide-gated (HCN) channel. We also confirmed that Ang II acutely enhanced HCN currents in primary cultured NG neurons via NADPH-superoxide pathway. In this study, we examined the chronic effect of Ang II on the protein expression of HCN channels in the primary cultured NG neurons isolated from rats. Using immunofluorescent staining, we found that HCN1 was expressed in the A-type NG neurons, and HCN2 was expressed in the C-type NG neurons. Chronic treatment of Ang II (100 nM, 12h) induced protein expression of HCN2 besides overexpression of HCN1 in the A-type NG neurons; and overexpression of HCN2 in the C-type NG neurons. In addition, whole-cell patch clamp data revealed that chronic treatment of Ang II significantly increased the density of HCN currents in A- and C-type NG neurons. Losartan (an AT₁ receptor antagonist, 1µM) markedly inhibited the effect of 1 Ang II on the density of HCN channels. These results suggest that Ang II not only acutely modulates the electrophysiological properties of HCN channels, but also chronically induces the protein overexpression of HCN channels.
ROLE OF ANGIOTENSIN II RECEPTORS IN NEURONAL STEM CELLS PROLIFERATION

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Our previous study demonstrated that, in both rats and mice, angiotensin II (Ang II) type 2 receptor (AT2R) expression in the brain is lower in the fetus and neonate than in adults, and that Ang II type 1 receptors (AT1R) exhibit an opposite expression profile, suggesting an involvement of a potentially important functional role for AT1R and AT2R in brain developmental processes, such as neurogenesis. Neurogenesis relies upon the proliferation, differentiation and migration of neural stem cells (NSCs) which are capable of self-renewal and are multipotent. However, whether AT1R and AT2R regulate NSCs proliferation remains unknown. Using Western-blotting and immunocytochemistry, from 0 to 8 day cultured NSCs, both AT1R and AT2R expression elicited an increase pattern. Ang II (100 nM) significantly increased NSCs proliferation (135%±12%) measured by CyQUANT cell proliferation assay. This proliferation was attenuated by the AT2R antagonist-PD123319 (97%±5%), but not AT1R antagonist-losartan (129%±4%). Furthermore, Ang II induced a time-dependent activation of ERK/MAPK and PI3K/Akt in NSCs, which play critical roles in the cell proliferation. Consistent with Ang II-NSCs proliferation data, the stimulatory effect of Ang II on ERK and Akt phosphorylation was attenuated by PD123319, but not losartan. These data clearly indicate that Ang II, contributes to NSCs proliferation via AT2R and activation of ERK and Akt.
ACTIVATION OF CENTRAL ANGIOTENSIN TYPE 2 RECEPTOR BY COMPOUND 21 SUPPRESSES SYMPATHETIC OUTFLOW IN CHRONIC HEART FAILURE RATS

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In previous studies, we demonstrated that, in normal rats, intracerebroventricular (icv) infusion of Compound 21 (C21) significantly decreased arterial blood pressure (BP) and norepinephrine (NE) excretion. In this experiment, we determined the effects of central treatment of C21 on sympathetic drive in rats with coronary ligation induced chronic heart failure (CHF). C21 was icv infused by a Micro-osmotic pump at a rate of 0.5ug/ul/2hrs for 7 days. During these days, BP and heart rate were continually recorded by a radio telemetry system to analyze arterial baroreflex function (ABR) under conscious condition. On day 7 the rats were anesthetized and renal sympathetic nerve activity (RSNA) was directly recorded to determine sympathetic tone and response to icv injection of Ang II. We found that: (1) C21 treatment reversed MAP decrease in CHF rats in both daytime and nighttime, and it slight decreased HR in CHF rats; (2) C21 treated rats exhibited a higher ABR gain than control rats in both sham and CHF states; (3) Under anesthetization, the baseline RSNA was significantly lower in C21 treated CHF rats than that in control CHF rats; (4) C21 treated CHF rats had lower sympahto-excitatory response to icv injection of Ang II than control did in both MAP and RSNA; (5) C21 treated CHF rats had more nNOS expression and less AT1R expression than vehicle treated CHF rats in SFO, PVN and RVLM. These results suggest that activation of central AT2R by C21 evoked sympahto-inhibition via improvement of arterial baroreflex function by suppressing central AT1R signaling in CHF rats.
MECHANISMS OF ANGIOTENSIN II TYPE 1 RECEPTOR DOWNREGULATION FOLLOWING EXERCISE TRAINING IN ANIMALS WITH HEART FAILURE.

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Although exercise training (ExT) is an important therapeutic strategy for improving quality of life in patients with chronic heart failure (CHF), the central mechanisms by which ExT is beneficial are not well understood. The Angiotensin II type 1 Receptor (AT1R) plays a pivotal role in the progression and severity of CHF, and as such is upregulated in a number of tissues. AT1R and other G Protein Coupled Receptors are marked for internalization and recycling via G Protein Coupled Receptor Kinase (GRK) phosphorylation. Because previous studies have shown that the beneficial effects of ExT rely on a reduction in Ang II and sympathetic nerve activity, we hypothesized ExT would decrease AT1R expression and the regulation of AT1R by GRK5 in the Paraventricular Nucleus (PVN) and Rostral Ventrolateral Medulla (RVLM) of rats with CHF. Animals were infarcted by coronary artery ligation and were exercised four weeks post-surgery on a treadmill at a final speed of 25 m/min for 60 minutes, 5 days a week for six weeks. Western blot analysis of PVN revealed a significant upregulation of both AT1R and GRK5 in the infarcted group, a trend that was reversed by ExT. Furthermore, the relative expression of phosphorylated AT1R and AT1R/GRK5 physical association was increased in the infarcted sedentary group, and reversed by ExT. The interaction of AT1R and GRK5 was examined further in cultured CATH.a neurons. Overexpression of GRK5 led to a blunted Angiotensin II-mediated upregulation of AT1R; conversely, silencing of GRK5 exacerbated Angiotensin II-mediated AT1R upregulation. Taken together, increased GRK5 may serve as a compensatory mechanism for AT1R upregulation in HF, and ExT mitigates the increase in both AT1R and its regulatory components.
ALTERATION IN SKELETAL MUSCLE AFFERENTS IN RATS WITH CHRONIC HEART FAILURE

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An exaggerated exercise pressor reflex (EPR) contributes to exercise intolerance and excessive sympatho-excitation in the chronic heart failure (CHF) state. However, the components of this reflex that are responsible for the exaggerated EPR in CHF remain unknown. To determine whether muscle afferents function is altered in CHF, we recorded the discharge of group III and IV afferents in response to static contraction, passive stretch and hindlimb intra-arterial injection of capsaicin in sham and CHF rats. We also investigated the roles of purinergic 2X receptor (P2X) and the transient receptor potential vanilloid 1 (VR1) in mediating the altered sensitivity of muscle afferents. Compared with sham rats, CHF rats exhibited greater responses of group III afferents to contraction and stretch whereas the responses of group IV afferents to contraction and capsaicin were blunted. Hindlimb intra-arterial infusion of pyridoxal phosphate-6-azophenyl-2’4-disulfonic acid, a P2X antagonist, attenuated the responses of group III afferents to contraction and stretch in CHF rats to a greater extent than in sham rats. Western blot data showed that P2X3 receptors were significantly upregulated in DRG of CHF rats whereas VR1 receptors were significantly downregulated. Immunohistochemical evidence showed that immunostaining of the P2X3 receptors was more intense in both IB4-positive (C-fiber) and NF200-positive (A-fiber) neurons in DRG of CHF rats whereas the immunostaining of the VR1 receptors was decreased in IB4-positive neurons. These data suggest that group III afferents are sensitized whereas group IV afferents are desensitized in CHF, which is related to the dysfunction of P2X and VR1 receptors.
ANGIOTENSIN II DECREASES EXTRACELLULAR SUPEROXIDE LEVELS IN A BRAIN MICROVASCULAR ENDOTHELIAL CELL CULTURE MODEL

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Angiotensin II (AngII), through its actions in the central nervous system, is involved in the regulation of blood pressure and body fluid homeostasis. Reactive oxygen species generated by both central neurons and brain microvascular endothelial cells (BMECs) mediate, at least in part, neurocardiovascular responses induced by AngII. However, the precise communication via paracrine signaling between brain microvascular endothelial cells and neurons in brain angiotensinergic signaling remains unclear. Here, we tested the hypothesis that AngII stimulation of BMECs increases extracellular superoxide (O$_2^-$) levels. Mouse BMECs were treated with AngII (100nM) and extracellular O$_2^-$ levels were measured by electron paramagnetic resonance (EPR) spectroscopy using the non-cell permeable spin probe PPH (600 mM). Surprisingly, AngII modestly decreased extracellular O$_2^-$ levels within 4 min and up to 30 min of stimulation with a significant decrease in extracellular O$_2^-$ levels observed at 60 min of AngII stimulation versus vehicle (vehicle: 71000 ± 17000 EPR arbitrary units (a.u.) vs. AngII: 40000 ± 9800 EPR a.u., P<0.01). Incubating BMECs with extracellular superoxide dismutase (SOD), a specific O$_2^-$ scavenger, virtually abolished the EPR spectrum (28000 ± 13000 EPR a.u., P<0.05 vs. vehicle), thus indicating the specific detection of O$_2^-$.

The AngII-induced decrease in extracellular O$_2^-$ was significantly attenuated by pre-treating cells with the AT2 receptor antagonist, PD123319 (1µM) (76000 ± 16000 EPR a.u., P<0.01 vs. AngII). Pre-treating BMECs with the AT1 receptor antagonist, losartan (1µM), or a nitric oxide synthase inhibitor, L-NAME (100µM), modestly, but not significantly, inhibited the AngII-induced decrease in extracellular O$_2^-$ levels. Together, these data suggest that AngII decreases extracellular O$_2^-$ levels produced by BMECs and that these effects are mediated via AT2 receptor activation together, at least in part, with a nitric oxide-mediated mechanism. In conclusion, these studies establish the involvement of AT2 receptor signaling in regulating levels of extracellular O$_2^-$ produced by BMECs, which may be crucial for paracrine communication within the neurovascular unit.
ENHANCED ACTIVATION OF RVLM PROJECTING PVN NEURONS IN RATS WITH CHRONIC HEART FAILURE

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Previous studies indicate that there is increased activation of the paraventricular nucleus (PVN) in rats with chronic heart failure (CHF), as measured/indexed by increased hexokinase or cFos activation. However, it is not clear if the pre-sympathetic neurons within the PVN are specifically overactive, and what is driving these neurons. Also, it is not known if they have altered responses to baroreflex or osmotic challenges? Experiments were conducted in rats with CHF 6-8 weeks after coronary artery ligation and with infarcts of >30% and left ventricular end-diastolic pressure (LVEDP) of >15 mmHg. In 160 spontaneously active neurons recorded in the PVN, 61 units were antidromically activated from the rostral ventrolateral medulla (RVLM). Baseline discharge rate of PVN-RVLM neurons in CHF rats was significantly greater than sham rats (6.0±0.6 vs. 2.7±0.3 spikes/s, \(P<0.05\)). Picoinjection of N-methyl-D-aspartate (NMDA) receptor antagonist DL-2-amino-5-phosphonovaleric acid (D-AP5) significantly decreased the basal discharge by 80% in CHF rats, compared with 37% in sham rats. Forty-nine percent of PVN-RVLM neurons responded to increases and decreases in the mean arterial pressure (MAP). The increases in discharge rate of PVN-RVLM neurons to a decrease in MAP were significantly attenuated in rats with CHF compared with sham rats (\(\Delta \% \) 52±8 vs. 192±65, \(P<0.05\)), but not to increases in MAP. A number of PVN-RVLM neurons (63%), were excited by internal carotid artery injection of 2.1osmol/L NaCl while 10% were inhibited. The increases in discharge rate of PVN-RVLM neuronal to hypertonic stimulation were enhanced in rats with CHF compared with sham rats (\(\Delta \% \) 131±17 vs. 91±12, \(P<0.05\)). Taken together, these data suggest that RVLM projecting PVN neurons are more active under basal conditions and this overactivation is mediated by an enhanced glutamatergic tone in rats with CHF. This enhanced activation of PVN-RVLM neurons may contribute to the altered responses to various autonomic cardiovascular challenges commonly observed during CHF.
Unlike the better characterized role of apoptosis and inflammation, the role of necrosis in cisplatin nephrotoxicity has not been defined. Here we demonstrate that cisplatin administration increased poly(ADP-ribose) polymerase (PARP1) expression and activation, which was accompanied by the development of kidney dysfunction. *Parp1* wild-type (WT) and knockout (KO) male mice were sacrificed 3 and 5 days after cisplatin intraperitoneal injection (single dose of 20 mg/kg body wt). The cisplatin treatment induced PARP1 expression and activation in WT kidneys, and its deletion showed a significant reduction in cisplatin-induced kidney dysfunction and tubular necrosis, but not apoptosis, compared with WT mice using *in vivo* and *in vitro* studies. Moreover, neutrophil infiltration and upregulation of proinflammatory genes including intracellular adhesion molecule-1 (ICAM-1), tumor necrosis factor-a (TNF-a), toll-like receptor 4 (TLR4) and various other cytokines/chemokines were markedly abrogated in *Parp1*-KO kidneys after cisplatin injury. Activation of nuclear factor-κB (NF-κB), c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK), associated with cisplatin-induced injury in WT kidneys, was notably abolished in *Parp1*-KO kidneys. Pharmacological inhibitor (PJ34, 10 mg/kg body wt, ip beginning 24 h before cisplatin injection twice daily until euthanasia) of PARP1 protected against cisplatin-induced kidney structural and functional damage and proinflammatory protein induction, similarly to that in *Parp1*-KO kidneys. Taken together, our findings suggest that loss of PARP1 protects against cisplatin-induced tubular necrosis and NF-κB/MAPK-dependent inflammation. Targeting PARP1 may offer a potential therapeutic strategy for cisplatin-induced nephrotoxicity.
CONTRIBUTION OF OXIDATIVE STRESS AND INFLAMMATION ON CAROTID BODY CHEMOSENSORY POTENTIATION INDUCED BY INTERMITTENT HYPOXIA

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Chronic intermittent hypoxia (CIH), a main feature of obstructive sleep apnea, enhances carotid body (CB) chemosensory responses to acute hypoxia. The CIH-induced chemosensory potentiation has been attributed to increased levels of reactive oxygen species (ROS) in the CB, but it is matter of debate if ROS perse may increase the CB chemosensory discharges. We found that CIH induced a ROS-dependent increases of TNF-α and IL-1β within the CB, suggesting that these pro-inflammatory cytokines may mediate the ROS-induced potentiation. Thus, we studied the effect of ibuprofen on the CB chemosensory responses to acute hypoxia, as well as on the basal chemosensory activity recorded in normoxia. Male Sprague-Dawley rats were treated with ascorbic acid (AA 1.25g/L) or ibuprofen (IB, 50mg/kg) and were exposed to CIH (5% O\textsubscript{2}, 12 times/hr for 8 hr) or sham conditions for 21 days. Rats were anaesthetized with sodium pentobarbitone 40 mg/kg, and the chemosensory discharges were recorded from the carotid sinus nerve in response to several levels of inspired O\textsubscript{2} (FiO\textsubscript{2} 5-100%). The CBs were extracted from anaesthetized rats and fixed in buffered paraformaldehyde 4% to measure the immunohistochemical levels of TNF-α and IL-1β. CIH enhanced both basal CB discharges and chemosensory responses to hypoxia, and increased TNF-α and IL-1β in the CB without changing the number of ED-1 positive cells in the tissue. Treatment with AA or IB prevented the cytokines overexpression in the CB. Ibuprofen reduced the enhanced CB basal discharge, but did not prevent the enhanced chemosensory responses to acute hypoxia induced by CIH, while AA prevented both the enhanced basal discharges and the chemosensory responses to acute hypoxia. Thus, our results suggest that IL-1β and TNF-α does not mediate the enhance the CB chemosensory responses to hypoxia, but contributes to increase the basal CB chemosensory discharges measured in normoxia in rats exposed to CIH.
POSTER P-10

NITRIC OXIDE INHIBITS THE EXPRESSION OF AT₁ RECEPTORS IN NEURONS

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We have previously observed an increased expression of angiotensin II type 1 receptor (AT₁ R) with enhanced AT₁ R-mediated sympathetic outflow and concomitant down-regulation of neuronal nitric oxide synthase (nNOS) expression with reduced nitric oxide (NO) mediated inhibition from the PVN in rats with heart failure. It is however, not clear if the decreased NO contributes to the increased expression of AT₁ R in neurons. To test the hypothesis that NO exerts an inhibitory effect on AT₁ R expression in the PVN, we used primary cultured hypothalamic cells of neonatal rats and neuronal cell line NG108-15 as in vitro models. In hypothalamic primary culture, NO donor sodium nitroprusside (SNP) induced dose-dependent decreases in mRNA and protein of AT₁ R (10⁻⁶ M SNP, AT₁ R protein was 10±2% of the control level) while NOS inhibitor N⁵-monomethyl-L-arginine (L-NMMA) induced dose-dependent increases in mRNA and protein levels of AT₁ R (10⁻⁶ M L-NMMA, AT₁ R protein was 148±8% of the control level). Moreover, similar effects of SNP and L-NMMA on AT₁ R expression were also observed in NG108-15 cell line (10⁻⁶ M SNP, AT₁ R protein was 30±4% of the control level while at the dose of 10⁻⁶M L-NMMA, AT₁ R protein was 171±15% of the control level). Consistent with these observations, specific inhibition of nNOS, using antisense caused an increase in AT₁ R expression while over-expression of nNOS, using adenoviral gene transfer caused an inhibition of AT₁ R expression in NG-108 cells. Furthermore, down regulation of AT₁ R mRNA as well as protein level in neuronal cell line in response to SNAP (S-nitroso-N-acetylpenicillamine) treatment was blocked by the PKG (protein kinase G) inhibitor, 8-bromoguanosine-3′,5′-cyclic monophosphorothioate while peroxynitrite scavanger, deforxamine has no effect. These results suggest that NO act as an inhibitory regulator of AT₁ R expression and the activation of PKG is the required step in the regulation of AT₁ R gene expression via cGMP-dependent signaling pathway.
MECHANISM OF UPREGULATION OF ANGIOTENSIN-1-RECEPTOR IN CATH.A NEURONAL CELLS VIA A SYNERGISTIC ACTIVATION OF TRANSCRIPTION FACTORS CREB AND NFkB

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In a previous study we have shown that transcription factors nuclear factor kappa B (NFkB) and the Ets like gene-1 (Elk-1) modulate the expression of the angiotensin II type I receptor (AT1R) in a neuronal cell line (CATH.a) following Ang II stimulation. cAMP response element binding protein (CREB) is another transcription factor that has also been implicated in AT1R gene transcription. The purpose of this study was to examine the functional synergy between NFkB and CREB activation. We hypothesized that the transcription of the AT1R gene is upregulated by several cascades of transcription factor reactions involving NFkB/CREB and Elk-1. The synergistic role of CREB and NFkB in promoting AT1R gene expression was corroborated using siRNA-mediated silencing of CREB and NFkB. EMSA studies employing CREB and NFkB demonstrated increased protein - DNA binding as a result of Ang II stimulation which was diminished by siRNA silencing of p65 as well as CREB. Upstream inhibition of p38 MAPK (mitogen activated protein kinase) and the calmodulin kinase pathway using KN-62 (a highly selective inhibitor of rat brain calcium-calmodulin dependent protein kinase II), resulted in inhibition of both CREB and NFkB (p65). These findings suggest that Ang II may activate multiple signaling pathways involving the p38MAPK leading to the activation of NFkB and CREB resulting in the up regulation of the AT1R gene. This study provides insight into the molecular mechanisms involving multiple transcription factor activation in a coordinate fashion which may be partially responsible for the pathophysiology of clinical conditions associated with increased activation of the renin angiotensin system.
SIMVASTATIN TREATMENT ATTENUATES ENHANCED CAROTID BODY CHEMOREFLEX SENSITIVITY IN RATS WITH HEART FAILURE

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The carotid body chemoreflex (CBC) is enhanced in congestive heart failure (CHF) and contributes to increased sympathetic nerve activity (SNA). Impaired blood flow in CHF is associated with reduced nitric oxide (NO) and NOS levels in the carotid body (CB) and contributes to the enhanced CBC sensitivity. The endothelial transcription factor Krüppel -like factor 2 (KLF2) is regulated by shear stress and mediates transcription of endothelial NO S (eNOS). We hypothesized that SIM treatment would ameliorate the increases in CBC sensitivity and restore KLF2 protein levels in the CB and nucleus of the solitary tract (NTS) in a rodent model of CHF. Ventilatory responses to isocapnic hypoxia (IsoH) were measured to assess CBC sensitivity. Left ventricular (LV) function was quantified via echocardiography. No changes in ventricular function were observed following SIM treatment. Ventilatory responses to IsoH were augmented in CHF, and SIM treatment prevented these increases. CB and NTS KLF2 expression were reduced in CHF, and this was attenuated in SIM animals. Our findings suggest that SIM attenuates enhanced CBC sensitivity in CHF and that this is mediated in part by normalizing KLF2 and eNOS in the CBs and NTS.
CARBONYLATION IS AN UNDERLYING CAUSE FOR DYSREGULATION OF RYANODINE RECEPTOR FROM THE SARCOPLASMIC RETICULUM DURING DIABETES

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Dysregulation of cardiac ryanodine receptors (RyR2) is a principal cause for aberrant Ca\(^{2+}\) release from the sarcoplasmic reticulum (SR), heart failure (HF) and ventricular arrhythmias in individuals with diabetes mellitus. However, mechanisms underlying RyR2 dysregulation during diabetes remain undefined. This study assesses whether carbonylation (modification by reactive carbonyl species, RCS) is an underlying cause. After 8 weeks of diabetes, spontaneous Ca\(^{2+}\) release was enhanced and evoked Ca\(^{2+}\) release was dyssynchronous (non-uniform) in rat ventricular myocytes. RyR2 protein level remained unchanged but its activity became heterogeneous. Western blots and mass spectrometric analyses of RyR2 from diabetic animals revealed modification by RCS at select basic residues. Altering charge and increasing bulk on affected residues resulted in channels with low to exaggerated cytoplasmic Ca\(^{2+}\) responses, akin to that seen during diabetes. Expressing carbonylation-mimicking mutants in RyR2-null HEK-293T cells induced abnormal intracellular Ca\(^{2+}\) cycling. Exposing healthy rat ventricular myocytes to a prototype reactive carbonyl species methylglyoxal (MGO) triggered aberrant SR Ca\(^{2+}\) release, similar to that seen in diabetic myocytes. Low MGO also increased and high MGO decreased the open probability of RyR2. Treating diabetic rats with scavengers of RCS blunted carbonylation of RyR2 and aberrant SR Ca\(^{2+}\) release. From these data we conclude that modification by RCS is an underlying cause for RyR2 dysregulation during diabetes. Our data also suggest that reducing RCS levels may be potentially important for slowing heart failure progression in individuals with diabetes mellitus.
We earlier discovered that chloroform extracts of hog barn dust (HBD) perturbs intracellular Ca\(^{2+}\) homeostasis in skeletal muscle cells by binding to and modulating ryanodine receptor calcium release channel (RyR1), providing a possible mechanism (and/or a physiological basis) for chronic skeletal muscle weakness and fatigue reported by hog confinement facility workers. In this study we expanded the work to determine if HBD also bind to and modulate the activities of RyR2 and RyR3, the other RyR isoforms found in heart, brain, diaphragm and the vasculature. HBD collected from medium to large farms (5000-10,000) in Nebraska were extracted with phosphate buffered saline (1 g/10 ml for 2 hr), sterile filtered and separated by molecular weight using Sephadex beads. \([^{3}\text{H}]\)ryanodine binding, lipid bilayer and single cell confocal imaging assays were then used to assess the activity of the Sephadex fractions. In displacement binding assays, fraction 12 (F12, \(M_m <6000\) Da) was the most potent, displacing >70\% of \([^{3}\text{H}]\)ryanodine bound to RyR1, RyR2 and RyR3 (IC\(_{50}\) ~ 0.3-2.0 mg/ml). Interestingly, while its displacement curves from RyR2 and RyR3 were parallel to that of the prototype ligand ryanodine, its displacement from RyR1 was not parallel to that of ryanodine. In lipid bilayer assays, F12 decreased the open probability of RyR1, RyR2 and RyR3 in a dose-dependent manner (4 \(\mu\)g &ndash; 400 \(\mu\)g/ml). F12 (100-400 \(\mu\)g/ml) also lowered intracellular Ca\(^{2+}\) in C\(_{2}\)C\(_{12}\) skeletal muscle myotubes (selectively expresses RyR1) and rat left ventricular myocytes (selective for RyR2). From these data we conclude that aqueous extracts of HBD contain components that inhibit all three isoforms of RyR, resulting in a reduction in intracellular Ca\(^{2+}\). Since activation of RyR is needed for increasing intracellular Ca\(^{2+}\) following ligand and electrical stimulation to execute physiologic functions inhibition of RyR by HBD may help explain the multiple ailments reported by confinement facility workers. Since persistent activation of RyR1 and RyR2 results in dysregulated muscle contraction, these new data suggests a mechanism for that, at least in part, HDE exposure augments the effects of cardiovascular disease and musculoskeletal disorder via RyRs.
Neuron migration is an essential process of normal brain development. Defects in this process are associated with brain malformation and multiple neurodevelopmental disorders including mental retardation, autism, and lissencephaly. However, little is known about the regulatory mechanisms of neuronal migration in the developing brain. Here, using genetic manipulation in-utero, we show that regulation of GSK-3 activity is critical in radial neuronal migration in the developing dorsal telencephalon. Activation of GSK-3 suppresses the migration capacity of neurons. The regulatory activity of GSK-3 in radial neuron migration is mediated by mTOR signaling. GSK-3 activation decreases the levels of phospho-4EBP1 and phospho-S6, markers for mTOR activity, in cortical neurons. Additionally, GSK-3 directly interacts with a negative mTOR regulator, TSC2 in cortical neurons, suggesting that GSK-3 controls mTOR activity in migrating cortical neurons. Furthermore, knockdown of mTOR signaling components, Raptor and Rheb, using shRNA enhances neuronal migration, while TSC2 knockdown delays the process. Taken together, these findings suggest that GSK-3 plays an important role in radial neuron migration in the developing brain and that the mTOR pathway mediates the GSK-3 signal.
Calmodulin (CaM) is required for the activity of many cellular proteins yet is expressed insufficiently for all its targets. We hypothesized that HMG-CoA reductase inhibitors improve endothelial functions in part by modulating CaM-dependent activities. At low therapeutic doses, simvastatin upregulates CaM expression in primary endothelial cells by up to 250%. This effect is not affected by pretreatment with mevalonate, but is abolished by inhibition of Akt activity. The increase in CaM expression is associated with increased activity of the CaM-dependent plasma membrane Ca\(^{2+}\)-ATPase. Simvastatin phosphorylates the dominant CaM-binding protein eNOS at Ser-617, Ser-635 and Ser-1179, and purified Akt phosphorylates eNOS at the same residues. Simvastatin also upregulates CaM expression in HEK 293 cells, but to a higher extent in cells stably expressing eNOS, suggesting that the increase in CaM expression in endothelial cells is enhanced by the effect of simvastatin on eNOS. Simultaneous measurements of free Ca\(^{2+}\) and free Ca\(^{2+}\)-CaM showed that at the same free Ca\(^{2+}\) concentrations, HEK 293 cells expressing an eNOS mutant with phosphomimetic substitutions at Ser-617, Ser-635 and Ser-1179 produce higher free Ca\(^{2+}\)-CaM signals than cells expressing eNOS with unphosphorylatable substitutions. Spectro-fluorometric competitive binding assays using indo-1 and an apoCaM biosensor revealed that the S617D/S635D/S1179D-eNOS mutant displays a 3-fold increase in the Ca\(^{2+}\)-sensitivity for complex formation with CaM compared to WT-eNOS, lowering the EC\(_{50}(\text{Ca}^{2+})\) value from 180 ± 2 nM to 60 ± 2 nM, a value well within the basal Ca\(^{2+}\) level in cells. Our data strongly indicate that at therapeutic doses simvastatin improves endothelial functions by increasing CaM expression and CaM-dependent functions via activation of Akt and subsequent phosphorylation of the dominant CaM-binding protein eNOS, leading to increases in its Ca\(^{2+}\)-CaM sensitivity. Our data also suggest a general mechanism that cellular CaM expression is governed in part by its requirement at basal Ca\(^{2+}\) concentrations in cells.
REGULATION OF ENDOTHELIAL Ca²⁺ INFLUX AND EFFLUX BY 17ß-ESTRADIOL

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17ß-estradiol (E²) exerts many cardioprotective effects through its actions on the vascular endothelium. E² is known to trigger acute Ca²⁺ signals in endothelial cells. However, the chronic effect of 17ß-estradiol on Ca²⁺ homeostasis and the underlying mechanisms are completely unknown. Here we have examined the main components of Ca²⁺ signaling in primary endothelial cells under chronic treatment with physiologic concentrations of E². We observed a ~30% reduction in the activity of the calmodulin (CaM)-dependent plasma membrane Ca²⁺-ATPase (PMCA) in cells chronically treated with E². In contrast, CaM binding to PMCA is increased by 50% while total PMCA expression is not affected. Inhibition of Src kinase activity prevents tyrosine phosphorylation of PMCA and reverses the effects of E² on PMCA activity, increasing it by 54%. This treatment, however, did not affect the increase in CaM binding to PMCA caused by E². Thus Src inhibition apparently reveals the effect of E² on CaM binding to PMCA. Inhibition of PKA activity substantially decreases PMCA activity in cells treated with or without E². Store-operated Ca²⁺ entry (SOCE) determined by the Mn²⁺ quenching technique is increased by ~20% in cells chronically treated with E². Our results demonstrate that E² increases agonist-induced Ca²⁺ signals in primary endothelial cells by increasing store-operated Ca²⁺ entry and decreasing Ca²⁺ efflux via the PMCA. The latter is mechanistically due to a combination of enhanced Src-dependent PMCA phosphorylation and increased PMCA-CaM binding, with the inhibitory effect of tyrosine phosphorylation being predominant.
IN VolVEMENT OF EPITHELIAL SODIUM CHANNEL (ENAC) IN DECREASED BAROREFLEX SENSITIVITY IN HEART FAILURE RATS

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Epithelial sodium channels (ENaC) can be served as mechanosensor in neurons and their terminals. Here we investigated the role of ENaC on aortic baroreceptor and baroreflex sensitivity in sham and CHF rats. CHF was induced by left coronary artery ligation. Immunofluorescent data indicated that β-ENaC and γ-ENaC were expressed in nodose neurons and the nerve ending in the aortic arch. Western blot data showed that CHF reduced β-ENaC and γ-ENaC protein expression in nodose neurons. In addition, using the whole cell patch-clamp technique, we found that mechanical sensitive current density of the aortic baroreceptor neurons were lower in CHF rats than that in sham rats. Depressor nerve and aortic baroreflex sensitivity to blood pressure were blunted in anesthetized CHF rats, compared with that in sham rats. These results suggest that reduced ENaC is involved in the attenuation of baroreceptor neuron sensitivity to mechanical stimulation, which subsequently contributes to the impairment of baroreflex in CHF state.
Teaching and Faculty Posters F1 – F5
Type 1 diabetes (T1D) triggers PKC-dependent NADPH oxidase (NOX) activation in the renal medullary thick ascending limb (mTAL), resulting in accelerated superoxide production. Superoxide is known to stimulate NaCl transport by the mTAL. Therefore, we hypothesized that T1D increases mTAL Na transport via PKC- and NOX-dependent mechanisms. As active Na transport is the primary renal ATP- and oxygen-consuming process, an oxygen-sensitive fluoroprobe was used to measure oxygen consumption (QO2) by mTALs from rats with STZ-induced T1D and Sham rats (n = 8 per group). In Sham mTALs, total QO2 was 0.34 ± 0.03 normalized relative fluorescence units (NRFU)/min/mg protein. The Na-K-ATPase (NKA) inhibitor ouabain (2 mM) reduced QO2 by 69 ± 4%, while inhibition of the Na-K-2Cl cotransporter (NKCC2) with 500 μM furosemide reduced QO2 by 58 ± 8%. Total QO2 by STZ mTALs was 0.74 ± 0.07 NRFU/min/mg protein (P < 0.05 vs Sham), reflecting increases in both ouabain-sensitive and furosemide-sensitive QO2. These observations indicate increased NKCC2- and NKA-dependent Na transport by the mTAL during T1D. Some mTALs from each rat were treated 30 min with NOX and PKC inhibitors prior to quantifying QO2. NOX inhibition (100 μM apocynin) significantly reduced furosemide-sensitive QO2 by STZ mTALs from 0.47 ± 0.06 to 0.27 ± 0.07 NRFU/min/mg protein, a value that did not differ from untreated or apocynin-treated Sham mTALs (0.20 ± 0.03 NRFU/min/mg protein). The PKC inhibitor calphostin C (1 μM) decreased furosemide-sensitive QO2 in both groups, achieving values that did not differ between Sham (0.11 ± 0.02 NRFU/min/mg protein) and STZ (0.13 ± 0.04 NRFU/min/mg protein). Inhibition of PKC-alpha/beta (1 μM Gö6979) or PKC-delta (10 μM rottlerin) normalized furosemide-sensitive QO2 by STZ mTALs, with values averaging 0.16 ± 0.02 and 0.18 ± 0.03 NRFU/min/mg protein, respectively. Although Gö6979 and rottlerin also provoked moderate decreases in furosemide-sensitive QO2 by Sham mTALs, the resulting values did not differ significantly from those observed in STZ mTALs exposed to these agents. The PKC-beta inhibitor indolylmaleimide-1 (50 nM) had no effect in either group. Virtually identical inhibitory patterns were evident with regard to ouabain-sensitive QO2. Thus, T1D increases ouabain- and furosemide-sensitive QO2 (and, hence, NKA- and NKCC2-dependent Na transport) by the mTAL through a mechanism involving NOX, PKC-alpha and PKC-delta. This phenomenon likely reflects, at least in part, PKC-dependent NOX activation which increases production of superoxide (known to stimulate Na transport by the normal mTAL); however, the mechanism through which superoxide activates NKCC2 and NKA during T1D remains to be determined.
We previously generated a unique mouse model of increased brain renin-angiotensin system (RAS) activity that exhibits hypertension (HT), polydipsia, and elevated basal metabolic rate. Adrenal steroids, sympathetic nervous activity (SNA) to kidney and adipose tissue, and plasma vasopressin (AVP) levels are elevated in these animals, whereas there is a suppression of the peripheral/circulating RAS. This double-transgenic model, termed sRA, expresses human renin from the neuron-specific synapsin promoter and human angiotensinogen via its own promoter, which targets both glia and neurons. sRA mice also exhibit the same cardinal features of the DOCA-salt model of HT. Based on these similarities, we hypothesized that HT in sRA mice is mediated by AVP-, SNA-, and endothelin-1- (ET-1) dependent mechanisms. Structurally, mesenteric arteries exhibited eutrophic inward remodeling (wall: control 17.3±0.6, vs sRA 26.8±1.6 μm P<0.01; lumen: 209.8±19.5 vs 159.0±11.0 μm P<0.05). Functionally, they exhibit a reduced response to KCl (100 nM: 73.1±1.2 vs 59.7±4.1 % max contraction, P=0.01), slightly increased sensitivity to ET-1 (EC50: 1.68±0.43 vs 0.45±0.17 nM, P<0.05) despite no change in ETA receptor mRNA (1.00 [0.92-1.09] vs 0.71 [0.58-0.87] fold±sem, P=0.18), and a major suppression of AVP efficacy (max response: 52.5±3.2 vs 17.3±6.5 % max contraction, P=0.001) likely due to suppressed V1A receptor mRNA (1.00 [0.86-1.17] vs 0.38 [0.30-0.47] fold±sem, P=0.003), but no change in responses to phenylephrine. Abdominal aortic rings exhibited a similar, selective impairment in contraction to AVP (max: 290±30 vs 120±10 mg, P=0.001) but no changes in responses to phenylephrine, ET-1, prostaglandin F2α, or angiotensin II. Aortic responsiveness to sodium nitroprusside was normal, but responses to acetylcholine were suppressed (EC50: 130±35 vs 879±298 nM, P<0.05), as expected with chronic HT. Chronic subcutaneous infusion of the non-selective, non-peptide V1A/V2 receptor antagonist conivaptan (YM-087, 22 ng/hr, 10 days) into a separate cohort of animals also normalized the hypertension of sRA mice (baseline SBP 112.2±2.3 vs 126.6±5.3, P=0.01; conivaptan SBP 117.7±2.9 vs 113.1±4.1 mmHg, P=0.36; genotype X drug interaction P=0.03). Collectively, these data highlight the involvement of AVP in the HT of mice with elevated brain RAS activity. Ongoing studies are aimed at identifying the relative importance of vascular V1A receptors (and resulting vasoconstriction) versus renal V2 receptors (and resulting volume loading) in the HT of these animals.
NERVOUS SYSTEM-TARGETED EXPRESSION OF CGRP/RAMP1 RECEPTORS ENHANCES BAROREFLEXES AND OPPOSES ANGIOTENSIN-INDUCED HYPERTENSION

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We recently reported that transgenic mice with ubiquitous expression of human receptor activity-modifying protein 1 (hRAMP1), an obligatory calcitonin gene-related peptide (CGRP) receptor subunit, exhibit increased baroreflex sensitivity for control of heart rate (BRS-HR) and resistance to angiotensin II (Ang-II) induced hypertension [Hypertension 55(3):627-635, 2010]. The contributions of vascular vs. neuronal CGRP receptors to the favorable phenotypes could not be definitively determined. Thus, the goal of this study was to determine if mice with hRAMP1 selectively targeted to the nervous system by the Nestin promoter [J Neurosci 27(10):2693-2703, 2007] show increased BRS-HR and resistance to hypertension. BP and HR were measured by telemetry in hRAMP1 (n=11) and littermate control (n=10) mice, before and following 2 weeks of Ang-II infusion (osmotic pump, 1000 ng/kg/min). We assessed BRS-HR (sequence technique), baroreflex BP buffering capacity (fold-increase in the pressor response to phenylephrine after vs. before ganglionic blockade), and sympathetic vasomotor tone (depressor response to ganglionic blockade). In control mice, Ang-II infusion increased mean 24-hr BP and sympathetic vasomotor tone, and decreased BRS-HR and baroreflex BP buffering. These deleterious effects were abrogated in hRAMP1 mice. Interestingly, baroreflex BP buffering capacity was markedly enhanced in hRAMP1 mice before as well as during Ang-II (see table). Selective targeting of hRAMP1 to the nervous system was confirmed by RT-PCR and the absence of an enhanced vascular depressor response to CGRP in hRAMP1 vs. control mice.

<table>
<thead>
<tr>
<th></th>
<th>Control Baseline</th>
<th>Ang-II Baseline</th>
<th>hRAMP1 Baseline</th>
<th>hRAMP1 Ang-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean 24-hr BP (mmHg)</td>
<td>106±1</td>
<td>171±6*</td>
<td>104±2</td>
<td>129±6‡</td>
</tr>
<tr>
<td>Sympathetic Tone (ΔmmHg)</td>
<td>-43±5</td>
<td>-68±8*</td>
<td>-47±4</td>
<td>-42±8†</td>
</tr>
<tr>
<td>BRS-HR (ms/mmHg)</td>
<td>2.5±0.3</td>
<td>0.7±0.1*</td>
<td>2.3±0.1</td>
<td>1.7±0.2†</td>
</tr>
<tr>
<td>Baroreflex BP buffering</td>
<td>1.8±0.1</td>
<td>1.3±0.2*</td>
<td>3.1±0.1†</td>
<td>3.1±0.4†</td>
</tr>
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In summary: 1) Under basal conditions, baroreflex BP buffering capacity is enhanced in hRAMP1 vs. control mice whereas BRS-HR, sympathetic vasomotor tone and mean BP are normal; and 2) the effects of Ang-II including hypertension, increased sympathetic tone, and impaired baroreflex are abrogated in hRAMP1 mice. The results identify protective autonomic and antihypertensive actions of neuronal CGRP/RAMP1 receptors and encourage targeting these receptors for therapeutic benefit. (AHA, NIH, VA)
Osmoregulatory ability was assessed in ~70 populations of 10 species of semiterrestrial crabs (Uca) distributed along the Atlantic coast of Brazil between Amapá and Santa Catarina. In the laboratory, crabs were exposed for 5 days to media ranging in osmolality from 15 to 3550 mOsm kg H₂O⁻¹; hemolymph osmolality was then measured in 10-μL aliquots using a Wescor 5520 osmometer. Survivorship, lower- and upper median-effective osmolality (UC₅₀) and isosmotic concentration [ISO] were estimated in populations for which habitat osmolality was also measured. All species were excellent hypo-/hyperosmoregulators. Mean [ISO] was <600 mOsm kg H₂O⁻¹ in the lone oligosaline species, between 650 and 770 mOsm kg H₂O⁻¹ in the seven mesosaline species, and >800 mOsm kg H₂O⁻¹ in the two eusaline species. Intraspecific variation in [ISO] was significant only in U. rapax, emphasizing its importance as a physiological set-point. Although UC₅₀ varied intraspecifically in six species, habitat osmolality varied significantly for U. rapax and U. victoriana alone. Thus, intraspecific variation in UC₅₀ likely results from local osmotic acclimation. Since genetic variation appears to be minor in most Uca species and unstructured across populations, the lack of physiological variation in [ISO] reflects the ecophenotypic nature of UC₅₀.

Financial support provided by Fulbright Fnd., U of Iowa GRERC, FAFESP and CNPq.
DESIGN AND IMPLEMENTATION OF INQUIRY-BASED RESEARCH PROJECTS TIED TO WRITTEN RESEARCH REVIEWS IN AN UNDERGRADUATE PHYSIOLOGY COURSE

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The laboratory portion of Bio 355, Human Anatomy, has previously utilized the iWorx data acquisition system and associated labs as a major portion of its curriculum. While this approach can be useful for applying concepts from lecture, the step-wise nature of the protocols have not been effective in promoting student inquiry. In order to encourage the natural interest students have in human processes and to ease the anxiety that can come with learning new technology, the laboratory was redesigned to include literature exploration and a written literature review, design of an experiment related to the literature that integrates the functions of at least two body systems, analysis of the resulting data using simple statistics, and presentation of research projects in a seminar-style format. Teams completed their projects over the course of ten weeks. Three of these weeks included additional preparatory lessons in data acquisition and analysis. Inquiry projects spanned topics ranging from the impact of loneliness on cardiovascular function to the effects of cooling and compression on muscle performance. Projects utilized a range of methodologies, including the iWorx apparatus. From the previous year, students were more confident in using the iWorx equipment and were more motivated to complete their laboratory work. In addition, students were able to tie their projects in to current physiological research.
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