Ohio Physiological Society

27th Annual Meeting

Wright State University

October 26-27, 2012

Ohio Physiological Society
27th Annual Meeting
Wright State University
October 26-27, 2012

OPS 2012 Program

Friday, October 26
Wright State University's Boonshoft School of Medicine
Enter from Colonel Glenn Highway onto Center Road, park in lot 16 or 17

4:00-5:00 P.M. Registration – White Hall Lobby

5:00 P.M. Welcome and Plenary Lecture
Ghandi Auditorium, White Hall

Welcome – Lynn Hartzler, OPS President

OPS 2012 Plenary Lecture (sponsored by the American Physiological Society)
James W. Hicks, PhD, Professor, Ecology & Evolutionary Biology
Associate Vice Chancellor for Research, Research Administration

"Tales from the Heart: Form and Function of the Vertebrate Cardiovascular System"

6:15 P.M. Reception
White Hall Lobby

7:00 P.M. OPS Banquet
White Hall Lobby

Ohio Physiological Society
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OPS 2012 Program

Saturday, October 27
Wright State University's Boonshoft School of Medicine
Enter from Colonel Glenn Highway onto Center Road, park in lot 16 or 17

8:00-9:00 A.M.	Registration – White Hall Lobby Please set-up posters during registration period
9:00 A.M.	Welcome – Lynn Hartzler, OPS President New Investigator Highlights Ghandi Auditorium, White Hall
9:05 A.M.	Nadja Grobe, PhD, Wright State University
9:30 A.M.	Peter MacFarlane, PhD, Case Western Reserve University
9:55 A.M.	Yana Zavros, PhD, University of Cincinnati
10:20 A.M.	Peter Piermarini, PhD, The Ohio State University, OARDC
10:45 A.M.	Morning Break – White Hall Lobby
11:00 A.M.	Trainee Data Blitz Ghandi Auditorium, White Hall
Noon	Lunch – White Hall Lobby
1:00-2:30 P.M.	Poster Session 1 (odd numbered poster boards) – White Hall Lobby
2:30-4:00 P.M.	Poster Session 2 (even numbered poster boards) – White Hall Lobby
4:00 P.M.	Business meeting – Ghandi Auditorium, White Hall
4:30 P.M.	Award Presentations – Ghandi Auditorium, White Hall

Ohio Physiological Society
27th Annual Meeting
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OPS 2012 Plenary Lecture

James W. Hicks, PhD
University of California, Irvine

http://compphys.bio.uci.edu/hicks/Comparative_Physiology/About_Me.html

"Tales from the Heart: Form and Function of the Vertebrate Cardiovascular System"

As a proponent of the field of comparative and evolutionary physiology, Jim Hicks has articulated the power of the comparative approach to understanding physiological mechanisms while simultaneously gaining insights into ultimate causation, *i.e.* the evolutionary or adaptive significance of a physiological process or trait. Jim's research on the evolution of the vertebrate heart includes experimentation at multiple levels of biological organization from integrating molecular biological techniques with cellular and organismal physiology through morphological, biomechanical, biophysical, ecological and evolutionary approaches. As an experimentalist, Jim investigates the significance of intra-cardiac shunts in air-breathing vertebrates.

Jim's contributions have been recognized with numerous distinguished awards throughout his career. Jim is a fellow of the American Association for the Advancement of Sciences – AAAS, was awarded the August Krogh Distinguished Lectureship by the Comparative and Evolutionary Physiology Section of the American Physiological Society, and, most recently, was awarded an Honorary Doctorate from the University of Aarhus, Aarhus, Denmark – a long-time center of cardio-respiratory physiology, as well as numerous other awards.

In addition to his many research contributions, Jim has provided service to comparative physiology as Chair of the Comparative and Evolutionary Physiology section of the American Physiological Society (APS), Chair of Comparative Physiology and Biochemistry for the Society for Integrative and Comparative Biology (SICB), Editor-in-Chief of Physiological and Biochemical Zoology, to name only a few. Jim has provided research opportunities in his lab for many students and colleagues, and he has long supported the establishment of research collaborations with great success.

Featured Presentation

Novel mass spectrometric imaging for discovery of enzyme activity *in situ*

Nadja Grobe, Mariana Morris, David R. Cool, Khalid M. Elased

Department of Pharmacology and Toxicology, Wright State University Boonshoft School of Medicine, Dayton, OH

The numbers of diabetics worldwide are rapidly increasing and prevention of the disease has become a key research objective. In diabetic nephropathy, the renin angiotensin system (RAS) is dysfunctional partially due to activation of the vasoconstrictor angiotensin (Ang) II. Angiotensin converting enzyme 2 (ACE2) is highly expressed in the kidney and has been shown to exerts its renoprotection via conversion of Ang II to the vasodilator Ang-(1-7). We developed a novel mass spectrometry (MS) imaging method in order to better understand the tissue distribution and activity of enzymes involved in Ang II processing. Mouse kidney sections (12 µm) were incubated with 10-1000 µmol/L Ang II for 5-15 min at 37 ºC. The formed peptides, Ang III and Ang (1-7), were identified by MALDI-TOF/TOF. Enzymatic processing of Ang II was dose and time dependent and absent in heat-treated kidney sections. Distinct spatial distribution patterns (pseudocolor images) were observed for the peptides. Ang III was localized in renal medulla while Ang (1-7) was present in cortex. Regional specific peptide formation was confirmed using microdissected cortical and medullary biopsies. Renal medullary Ang III generation was blocked by aminopeptidase A inhibitor, glutamate phosphonate. Cortical Ang (1-7) formation was reduced by ACE2 inhibitor, MLN-4760, and prolyl carboxypeptidase (PCP)/prolyl endopeptidase (PEP) inhibitor, Z-pro-prolinal. Using genetic mouse models deficient either in ACE2, PCP or PEP, the contribution of these peptidases to renal Ang II processing was explored. We found that ACE2 is the responsible enzyme for renal Ang II processing under neutral and basic conditions, while PCP catalyzes this reaction at acidic pH. This is the first report to demonstrate renal Ang II processing to Ang-(1-7) is independent of ACE2. Elucidating alternative Ang II processing mechanisms will aid toward understanding the complex role of the protective arm of the RAS in establishing renal and cardiovascular health.

Featured Presentation

Using a rodent model to mimic some of the postnatal profiles of hypoxia exposure experienced by preterm infants.

Jingning Ao, Catherine A. Mayer, Peter M. MacFarlane

Dept. of Pediatrics, Case Western Reserve University, Rainbow Babies & Children's Hospital

Chronic intermittent hypoxia (CIH) is associated with apnea of prematurity (AOP), whereas the premature transition into a hyperoxic air breathing environment can frequently lead to other O_2-related morbidities including retinopathy. Recent clinical trials have aimed to minimize the severity of retinopathy by maintaining preterm infants at a reduced level of O_2 saturation (termed, the "*low" target group*) for the first postnatal months. A disconcerting finding, however, was an increase in infant mortality indicating a heightened level of vulnerability in those infants that experience continuous hypoxemia together with CIH. Therefore, we developed a rodent model to closely mimic the *"low" target group* of preterm infants by exposing neonatal rats to both sustained (SH) and chronic intermittent hypoxia and assessed their effects on the respiratory neural control system. Using whole-body plethysmography, the magnitude of the hypoxic ventilatory response (HVR) following 10d of CIH (5% O_2/5 minutes, 8hrs/d) was unchanged (58.8±6.6% above baseline) at postnatal (P) age P16 compared to normoxia raised rats (48.9±6.4%). In contrast, the HVR was absent (10.8±5.4%) in rats pretreated with SH (11% O_2, 5d) prior to CIH, whereas SH alone did not affect the HVR. In summary, postnatal exposure to SH and CIH exert a synergistic disturbance in the mechanisms sub-serving the ventilatory response to acute hypoxia. We also provide evidence that neonatal SH exposure prior to CIH impairs the *in vitro* carotid chemoreceptor sensory response to acute hypoxia, despite significant compensatory responses in the nTS. We propose that sequential exposure to SH and CIH profoundly disturbs the peripheral chemoreceptor sensitivity to hypoxia which could contribute to the increased vulnerability of the *"low" target group* of preterm infants. These data could provide useful insight into the importance of avoiding SH in the intensive care of preterm infants during early and vulnerable periods of postnatal development.

Featured Presentation

Sonic Hedgehog: Understanding the role of a developmental morphogen in adult tissue

Yana Zavros
University of Cincinnati

Eradication of *Helicobacter pylori* correlates with regeneration of the gastric epithelium, ulcer healing and re-expression of the gastric morphogen Sonic Hedgehog (Shh). Ulcer healing is a complex process that is not completely understood in the stomach, but involves inflammatory cell infiltration, cell proliferation, migration, re-epithelialization and angiogenesis. Interestingly, Shh regulates multiple events essential for wound healing in other tissues including the lung, bone, heart and cornea. The immune response is crucial for repair in tissues that include the kidney and skin. In particular, macrophages are key immune cells that secrete necessary cytokines, chemokines and pro-angiogenic factors that are necessary regulators for repair. Shh is known to act as a monocyte/macrophage chemoattractant during repair of the myocardium and *H. pylori* infection. However, whether Shh signaling regulates regeneration of the epithelium by regulating the immune response during tissue repair in the stomach is unclear. Part of our research focuses on identifying the role of Shh as a regulator of gastric epithelial regeneration during wound healing. Using a mouse model expressing a parietal cell-specific, tamoxifen-inducible deletion of Shh (PC-iShhKO) we show that re-expression of Shh contributes to gastric regeneration. Our current study has clinical implications given that eradication of *Helicobacter pylori* correlates with re-expression of Shh, regeneration of the gastric epithelium and ulcer healing.

Featured Presentation

The molecular physiology of renal potassium channels in mosquitoes

Piermarini PM, Rouhier MF, Denton JS, and Beyenbach KW.

The Ohio State University, Vanderbilt University, and Cornell University

The 'kidneys' of mosquitoes are the Malpighian tubules. In contrast to mammalian kidneys, Malpighian tubules generate urine via active transepithelial secretion. The resulting urine is isomotic to the hemolymph and consists primarily of NaCl and KCl. Previous studies by our group have implicated barium-sensitive K^+ channels as a major route for the uptake of K^+ across the basolateral membrane of the Malpighian tubule epithelium in mosquitoes. In the present study, we aim to determine if inward-rectifying K^+ (Kir) channels contribute to the production of urine by mosquitoes. We reveal that the Malpighian tubules of the mosquito *Aedes aegypti* are characterized by the expression of three Kir channel subunits: *Ae*Kir1, *Ae*Kir2B, and *Ae*Kir3. Heterologous expression of these subunits in *Xenopus* oocytes demonstrates that the *Ae*Kir1 and *Ae*Kir2B subunits each form functional, homomeric Kir channels that are blocked by barium. In contrast, the *Ae*Kir3 subunit does not form functional, homomeric Kir channels. In isolated *Aedes* Malpighian tubules, the cation permeability sequence of the basolateral membrane mirrors that expected for Kir channels. Furthermore, the injection of a small-molecule inhibitor of the *Ae*Kir1 channel into the hemolymph of mosquitoes inhibits their ability to excrete urine. Taken together, the above data indicate that Kir channels in Malpighian tubules play an important role in the excretory physiology of mosquitoes. Funding is provided by a grant from the Foundation for the NIH, VCTR program.

Abstracts for Poster Presentations

Poster Presentations
Please hang your posters in White Hall Lobby during the registration period (8:00-9:00 A.M.). Posters can be taken down at the end of the second poster session at 4:00 P.M. Odd numbered posters present 1:00-2:30 P.M. and even numbered posters present 2:30-4:00 P.M.

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2	**Does chronic cold exposure prevent development of pathological hypertrophy in mice?** Spencer W. Barnhill and Paul J. Schaeffer
3	**Effects of chronic swim stress on cardiac function in Angiotensin II AT1a Receptor knockout mice** Najat Almahroug, Ahmad Alhajoj, Mahmoud Alghamri, Roberta L Pohlman, Lynn Hartzler, Mariana Morris
4	**Sarcolipin is a novel regulator of muscle based thermogenesis and metabolism in mammals** Naresh Chandra Bal, Santosh Kumar Maurya, Danesh H Sopariwala, Sanjaya Kumar Sahoo, Sana A Shaikh, Meghna Pant, Leslie A Rowland, Muthu Periasamy.
5	**Sarcolipin (SLN), a novel regulator of the Sarco-endoplasmic reticulum calcium (SERCA) pump, uncouples Ca^{2+} transport from ATP hydrolysis thereby causing futile Ca^{2+} cycling** Santosh Kumar Maurya, Muthu Periasamy
6	**Effects of an Eternal Energy Shot on Systolic Blood Pressure, Diastolic Blood Pressure, Pulse Pressure, and Heart Rate** Jonathan Price, Hillary Hassink, and Cathy L. Pederson
7	**Minimal exercise, maximal benefits for Type 2 diabetes** Kathleen Broomal, Avi Milgrom
8	**Normalizing hyperglycemia with insulin will reduce albuminuria by increasing ACE2 in Akita diabetic mice** Esam Salem, Khalid Elased
9	**Fructose and salt rich diet from weaning to adulthood: effects on autonomic modulation in rats** Romario Pacheco Andrade; Mauricio Di Fulvio; Vera de Moura Azevedo Farah
10	**High Fat Diet Induced Downregulation of Prolyl Carboxypeptidase in ACE2 Knockout Mice** Orly Leiva, Nathan M. Weir, Susan B. Gurley, Valerie Neff, Khalid Elased, Mariana

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11	**Effects of physical training in cardiac autonomic control rats submitted to a diet rich in fructose** Nunes, J.P.; Farah, D; Sartori, M.; Fiorino, P.; De Angelis, K.; Morris, M.; Farah, V.
12	**Preconditioning by electrical stimulation (EleS) induces cytoprotective effect on cardiac stem cells (CSCs)** Kim Sun Wook, Muhammad Ashraf
13	**THE OPERATING MECHANISM OF A NEW JAK3-FES-PLD2 SIGNALING PATHWAY THAT MEDIATES CELL GROWTH** Qing Ye, Julian Gomez-Cambronero
14	**Apelin regulation of potassium chloride cotransport (KCC) by the nitric oxide (NO) pathway in vascular smooth muscle cells (VSMCs) and effect of osmolality changes.** Neelima Sharma, Peter K Lauf, Norma C. Adragna
15	**The Flavonoid Apigenin is an inhibitor of PLD2 mediated proliferation and invasiveness in breast cancer cell line MDA-MB-231** Qing Ye, Samuel Kantonen, Julian Gomez-Cambronero
16	**Selective targeting of T cell subsets with functionalized lipid nanovesicles** Peter Hajdu, Tyler Thompson, Yeoheung Yun, Laura Conforti
17	**A Novel Approach for Localization of Gold Nanoparticles in Mice Central Nervous System** Fahimeh Fallahi, Saber M. Hussain, Ioana E. Pavel, James O'Callaghan, Monita Sharma, Ryan Saadawi, Mariana Morris
18	**Regulation of Coxsackie and Adenovirus Receptor (CAR) by Interleukin-8.** Poornima Kotha, Trisha Brockman, Katherine J. D. A. Excoffon
19	**Identification of Early Sepsis Biomarkers by using Dynamics in Biological Networks** Gopinath Sundaramurthy and Hamid Eghbalnia
20	**Adenosine inhibits human T lymphocyte migration via inhibition of KCa3.1 channels** Ameet A. Chimote, Vladimir Kucher, Nina Boiko, Zerrin Kuras, Peter Hajdu, Orsolya Szilagyi and Laura Conforti
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26	**Site of action of the inhibitors FIPI and NOPT on PLD2** Ramya Ganesan, Julian Gomez Cambronero
27	**Identification of the Catalytic Site of Phospholipase D2 (PLD2) Newly Described GEF Activity** Madhu Mahankali, Julian Gomez-Cambronero
28	**The impact of FoxO1 on skeletal muscle protein synthesis** Rachael A. Potter, Evan E. Schick, and Thomas J. McLoughlin
29	**Ang II/Ang (1-7) balance regulates the function and gene expression of cerebral endothelial cells** Xiang Xiao, Cheng Zhang, Ji Chen, Wenfeng Zhang, Yanfang Chen
30	**Angiotensin Converting Enzyme 2 Priming Enhances the Function of Endothelial Progenitor Cells and their Therapeutic Efficacy** Ji Chen, Xiang Xiao, Shuzhen Chen, Cheng Zhang, Jianying Chen, Yi Dan, Vinayak Shenoy, Mohan K Raizada, Bin Zhao, Yanfang Chen
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33	**Assessing the effect of shear stress on Aquaporin 1 expression in vascular endothelial cells *in vitro*.** Mollie Walton, Kyle McGrail, Carissa M. Krane
34	**Aquaglyceroporin expression and erythrocyte omoregulation in cultures from the freeze tolerant anuran, Cope's gray tree frog, *Hyla chrysoscelis*** Venkateshwar Mutyam, James Frisbie, David L. Goldstein, Wiebke S. Diestelkamp, Carissa M. Krane
35	**Permeabilities to water and glycerol differ in erythrocytes from freeze-tolerant *Hyla chrysoscelis* and freeze-intolerant *Lithobates catesbeiana*** Naava Honer, Luiz Finatti, Kara Bobka, James Frisbie, David Goldstein
36	**Cell swelling induced by water and glycerol gradients in hepatocytes from warm- and cold-acclimated *Hyla chrysoscelis*, a freeze-tolerant amphibian.** Ponti Tsou, Geetha Koneru, James Frisbie, David Goldstein
37	**Membrane trafficking of aquaglyceroporin HC3 in erythrocytes from the freeze tolerant anuran, Cope's gray treefrog, *Hyla chrysoscelis*** Elizabeth Wetzel, Philip Nickell, Venkateshwar Mutyam, James Frisbie, David L. Goldstein, Carissa M. Krane
38	**Aquaporin 4 expression and distribution during osmotic brain edema and following chronic treatment of desipramine** Robinson, Sergei Alexander, James E. Olson
39	**Purinergic receptors are critical for hypoosmotic volume regulation of 1321N1 human astrocytoma cells** J. E. Olson, M. Bradshaw

40	**Title: ACE2/Ang1-7 pathway protects brain from OGD-induced cell swelling and death by counteracting the Ang II/AT1 axis and in an age-dependent manner** Jiaolin Zheng , Guangze Li, Michelle Durrant, James Olson, Yulan Zhu and Yanfang Chen
41	**Characterization of stimulation-induced volume changes in the Ca1 region of rat hippocampus slices** Amanda Gutwein and James Olson
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43	**Membrane trafficking produces distinct β-adrenergic signaling for Cl^- secretion and K^+ secretion in guinea pig distal colonic mucosa** Susan Halm, Jin Zhang, and Dan Halm
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50	**Magnesium sensitivity of TRPM7 channels in leukocytes** Chokshi, R., Hourani, S., Kozak, J.A.
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57	**Use of DTI for Quantification and Analysis in Neuroimaging Data** Kevin Hatcher
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60	**Chemosensitive signaling of locus coeruleus neurons in the Bullfrog, *Lithobates catesbeianus*** Joseph Santin, Lynn Hartzler
61	**Hypercapnia Inhibits a Transient K^+ Current in Chemosensitive Neurons from the Nucleus Tractus Solitarius (NTS) of Neonatal Rats** Ke-yong Li and Robert W. Putnam
62	**Hypercapnia Inhibits Both Transient and Sustained Potassium Currents in Chemosensitive Neurons from Neonatal Rat Locus Coeruleus (LC)** Robert W. Putnam and Ke-yong Li
63	**Hands-on PhUn week activities at Medway Elementary School** Lynn Hartzler

Effect of body size on midgut morphology and transporter expression in *Manduca sexta*.

Gillen, Christopher M.; Yeoh, Aaron J.; and Vela-Mendoza, Allison V.

Department of Biology, Kenyon College, Gambier, OH 43022

Metabolic rate of *Manduca sexta* larvae scales with an exponent above 0.90, much greater than the 0.67 and 0.75 exponents predicted by geometric and fractal scaling of exchange surfaces. To test the hypothesis that midgut surface area scales differently than predicted, we measured mass of *Manduca sexta* midguts and midgut contents. Wet mass of gut tissue increased in comparison to total body mass with a scaling exponent of 0.85, while gut content mass scaled at a 1.33 exponent. Exponents for anterior and posterior midgut were 0.96 and 0.85, above the 0.67 value predicted for isometric scaling, while the exponent for middle midgut was 0.70. Together, these results suggest that surface area may increase more than predicted by theoretical models, thereby at least partly compensating for declining surface area as larvae grow. We also tested the hypothesis that increased expression of membrane proteins helps compensate for the decreased relative surface area of the midgut in later instars. We compared mRNA expression of KAAT1 – a potassium-amino acid transporter expressed on the apical membrane of *Manduca sexta* midgut – between 4th and 5th instar larvae. Expression of the KAAT1 gene was 2.3 to 3.1 fold higher in 5th compared to 4th instar larvae, supporting the hypothesis of increased membrane protein expression in larger larvae. We are optimizing RNA interference to characterize the consequences of decreased KAAT1 expression. A plasmid construct with a 453bp KAAT1 PCR fragment and flanking T7 promoters was developed for the production of KAAT1 dsRNA. We are testing the effects of different delivery methods, timing and dosage with assessment of phenotype and gene expression with qPCR.

Poster 2

Does chronic cold exposure prevent development of pathological hypertrophy in mice?

Spencer W. Barnhill and Paul J. Schaeffer

Dept. of Zoology, Miami University

The purpose of this study is to investigate the idea that prolonged cold exposure (which mimics exercise) can prevent the effects of pathological cardiac hypertrophy caused by obesity in mice. Given that exercise can reduce the detrimental effects of hypertrophic cardiomyopathy, it can be hypothesized that exercise may prevent the effects of obesity-induced pathological hypertrophy. Specifically, it is suspected that the physiological hypertrophy induced due to exercise will be the primary reason for the prevention in the onset of pathological cardiac hypertrophy in a mouse model of obesity. It is expected that physiological hypertrophy due to exercise will exhibit no dysfunction and will prevent the onset of pathological hypertrophy in obese mice subjected to exercise. In this experiment, three groups of mice were exposed to varying nutritional and environmental conditions. The control group included wild-type mice fed a standard chow diet, the second group included transgenic UCP-dta mice on a high-fat diet, and the third group included cold exposed transgenic UCP-dta mice on a high-fat diet. Gene expression analysis via PCR for ten different genes show increased expression for metabolic genes and their regulators in the experimental high-fat cold-exposed mice. These metabolic genes show a decrease in expression in the high-fat warm mice. Echocardiographic analysis showed relatively no difference between groups, but hints towards a development of heart failure in the cold-exposed group. Cardiac catheterization showed possible contractility impairment in the obese cold-exposed mice. This project provides insight into the structural, functional and metabolic phenotype of exercise-induced physiological hypertrophy and into how exercise influences the development of pathological hypertrophy. An understanding of the regulatory processes and interplay between physiological and pathological hypertrophy is needed in order to develop interventions capable of maintaining cardiac function.

Effects of chronic swim stress on cardiac function in Angiotensin II AT1a Receptor knockout mice

Najat Almahroug, Ahmad Alhajoj, Mahmoud Alghamri, Roberta L Pohlman, Lynn Hartzler, Mariana Morris

Department of Pharmacology & Toxicology, Department of Biological Sciences, Boonshoft School of Medicine, Wright State University

There is growing consensus that exercise training reduces cardiovascular risk. Moreover, exercise training has been recognized as an important and safe strategy for prevention and treatment of heart failure. Angiotensin II (AngII) through the activation of Ang type I receptor (AT1R) has patho-physiological relevance in cardiac remodeling. The goal was to assess the influence of chronic swimming stress on the cardiac function in AT1aR knockout mice. Adult male AT1R KO and wild type (WT), (15 weeks age, n=6/group) were used. The swim test was conducted for 60 minutes, 3 days a week for 7 weeks in 22-25°C water. Cardiac function was assessed by echocardiography (Echo), at week1 and at the end of the study, week7. Distance, velocity and maximum oxygen consumption {$VO2_{Max}$ (ml/min)} were recorded. Results showed that there was a significant higher ejection fraction (EF %) in AT1R KO exercise (AT1RKOEX) versus WTEX (75 % versus 55 %). Distance (cm) and Velocity (cm/s) significantly increased in AT1R KOEX as compared to WTEX, {$F (1, 32) = 9.35$, $p < 0.01$}. The ($VO2_{Max}$) analysis showed no differences between the groups (AT1R KO EX versus WTEX).The exercise produces a greater change in pumping capacity in AT1aRKO.

Poster 4

Sarcolipin is a novel regulator of muscle based thermogenesis and metabolism in mammals

Naresh Chandra Bal, Santosh Kumar Maurya, Danesh H Sopariwala, Sanjaya Kumar Sahoo, Sana A Shaikh, Meghna Pant, Leslie A Rowland, Muthu Periasamy.

Physiology and Cell Biology, College of Medicine, The Ohio State University, Columbus, OH

Sarcolipin (SLN) is a novel regulator of Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase (SERCA) pump and it is expressed exclusively in striated muscle. Recent *in-vitro* studies suggested that SLN can increase heat production by uncoupling SERCA-mediated ATP hydrolysis from Ca^{2+} transport but its role in muscle physiology has not been established. To demonstrate that SLN is the basis for muscle thermogenesis *in vivo*, we challenged $SLN^{-/-}$ mice to acute cold (4°C). Here we show that $SLN^{-/-}$ mice without interscapular Brown Adipose Tissue (*iBAT*) failed to maintain core body temperature (Tc, 37°C) and died when exposed to acute cold (4°C), whereas wildtype (WT) mice without *iBAT* were able to maintain Tc. Overexpression of SLN in the null background fully restored muscle-based thermogenesis, suggesting that SLN is the basis for SERCA-mediated heat production. We found that curare mediated blockade of shivering in WT mice without iBAT did not significantly compromise thermogenesis indicating existence of non-shivering thermogenesis (NST) in muscle. We show that Ryanodine receptor (RyR1)-mediated Ca^{2+} leak is an important mechanism for SERCA-activated heat generation. We also provide experimental data to suggest that SLN can interact with SERCA in the presence of Ca^{2+} and is thereby capable of promoting uncoupling of the SERCA pump and increasing heat production. We further demonstrate that loss of SLN predisposes mice to diet induced obesity, which suggests that SLN mediated NST is recruited during metabolic overload. These data collectively suggest that SLN is an important mediator of muscle thermogenesis and this mechanism could be recruited in energy metabolism.

Sarcolipin (SLN), a novel regulator of the Sarco-endoplasmic reticulum calcium (SERCA) pump, uncouples Ca^{2+} transport from ATP hydrolysis thereby causing futile Ca^{2+} cycling

Santosh Kumar Maurya, Muthu Periasamy

Physiology and Cell Biology, College of Medicine, The Ohio State University, Columbus, OH

Sarcolipin (SLN), a novel regulator of the Sarco-endoplasmic reticulum calcium (SERCA) pump, uncouples Ca^{2+} transport from ATP hydrolysis thereby causing futile Ca^{2+} cycling. By this process SLN increases ATP utilization by SERCA pump leading to increased energy expenditure. Therefore, in the presence of SLN, oxidation of cellular fuels in skeletal muscle (viz. glucose and fatty acids) must increase to maintain the optimum ATP level. We hypothesized that SLN can enhance energy expenditure leading to decreased fat accumulation. To validate this hypothesis, we challenged WT and $SLN^{-/-}$ mice on high fat diet (HFD). The results show that despite similar caloric intake $SLN^{-/-}$ mice gained more weight. MRI imaging and physical fat pad weighing showed that $SLN^{-/-}$ mice fed on HFD had increased fat accumulation. Interestingly, WT mice on HFD gained less weight but showed ~3 fold increase in SLN expression indicating a protective role of SLN against obesity. We found that phosphorylated-AMPK (the active form of AMP-Kinase) was decreased although total AMPK level was unchanged in skeletal muscles and adipose tissues of HFD fed $SLN^{-/-}$ mice suggesting a decrease in fat burning leading to increased fat accumulation. In contrast to $SLN^{-/-}$ mice, SLN^{OE} mice (SLN-overexpression which expresses ~5 fold higher SLN in all the skeletal muscles) consume more oxygen upon HFD feeding. Interestingly, the energy expenditure of HFD fed SLN^{OE} mice was significantly higher than WT mice indicating that SLN has the potential to utilize more calories. Further, we have observed that SLN^{OE} mice have better thermogenic ability during acute cold challenge. These results suggest that SLN plays an important role in diet as well as cold-induced thermogenesis. Therefore SLN is a key regulator of energy metabolism and the SLN-SERCA interaction might serve as a novel tool for treatment of obesity.

Poster 6

Effects of an Eternal Energy Shot on Systolic Blood Pressure, Diastolic Blood Pressure, Pulse Pressure, and Heart Rate

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The purpose of this study was to determine the effects of an energy shot on blood pressure and heart rate. A 2 oz. Eternal energy shot contains 2100 mg of an energy blend composed of mainly caffeine which causes bronchodilation and increases respiratory rate, blood pressure and heart rate for a few hours (Nurminen et. al, 1999). Blood pressure and heart rate were measured before consumption and at 15, 30, and 60 minutes after consumption of an Eternal energy shot. Before performing the experiment, information regarding age, heart health history, and previous caffeine and food consumption was collected. A paired sample T-test was used to detect differences from the baseline for all analyses. Increases in systolic blood pressure were significant at 15 minutes ($t(10) = -6.249$; $p = 0.001$) and 30 minutes ($t(10) = -14.362$; $p = 0.002$), but not significant at 60 minutes ($p > 0.05$). Increases in heart rate were significant at 30 minutes ($t(10) = -3.056$; $p = 0.030$), but not significant at 15 minutes and 60 minutes ($p > 0.05$). Changes in diastolic blood pressure and pulse pressure were not significant at 15 minutes, 30 minutes, or 60 minutes. The energy shot resulted in significant increases in systolic blood pressure at 15 and 30 minutes and heart rate at 30 minutes. Future studies should investigate the impact of different brands of energy shots up to four hours after consumption.

Minimal exercise, maximal benefits for Type 2 diabetes

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We present preliminary data in support of a novel exercise regimen based upon brief bouts of moderate intensity exercise that are strategically timed to moderate postprandial and all other blood glucose spikes. No exercise clothing or equipment is required; thus it can be readily integrated into a busy day in a "built environment" -- unlike other brief-interval regimens. The underlying premise, documented in the literature, is that muscle contractions can shunt blood glucose from the blood stream into muscle.

Thus far, our limited psychological and physiological findings are changing our thinking from linear causation of blood glucose excursions as an organizing principle to a multi-variable, systems view, for glycemic excursions.

Psychologically we are finding that the approach offers intrinsic rewards that make it readily amenable to habituation.

Physiologically we have evidence that eating many little meals is ill-advised, and that a daily period of modest fasting may "reset" the glucose metabolism set-point. Concerning the physiology of the approach itself, the shape of the "CGM" curve after the initiation of the exercise regimen is that of an exponential decay, suggesting a recruitment of forces within the physiological system, reinforcing both normoglycemia and glycemic stability.

We are currently pursuing funding with which to evaluate the effectiveness of the regimen in managing Type 2 diabetes in randomized studies. Proposed research will map HbA1c values against degrees of adherence to the regimen and will identify psychological and physical factors moderating adherence. The ultimate goal is to deliver the regimen to clinicians with guidelines for the selection of viable candidates.

Preliminary data was developed without outside funding.

KEY TERMS: Diabetes, Exercise, Blood Glucose

Poster 8

Normalizing hyperglycemia with insulin will reduce albuminuria by increasing ACE2 in Akita diabetic mice

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Diabetic nephropathy (DN) is a microvascular complication of diabetes that is clinically diagnosed by a progressive increase in albuminuria. Alterations within renin angiotensin system balance contribute to the pathogenesis of diabetic kidney disease. Angiotensin converting enzyme 2 (ACE2), a metallocarboxypetidase, has a renoprotective role due to its ability to form Angiotensin (1-7) [Ang-(1-7)] by degrading Angiotensin II (Ang II). Accumulating evidence shows that strict glycemic control attenuates diabetic kidney damage. Therefore, the aim of this study is to test the hypothesis that normalizing hyperglycemia with insulin will reduce albuminuria by increasing ACE2 in Akita diabetic mice. Type 1 diabetic Akita mice and their wild type (WT) littermates were used. Metabolic parameters were monitored weekly. Urine was collected over 24 hours to measure urinary albumin, total protein and ACE2 activity. Akita mice developed significant hyperglycemia compared to WT mice. There was a significant increase in urinary albumin excretion in Akita mice compared to WT mice. In addition, Akita mice demonstrated a significant increase in renal and urinary ACE2 activity compared to WT mice ($p<0.05$). Western blot revealed upregulation of renal ACE2 and downregulation of renal ACE protein expression in Akita mice compared to WT mice. Treatment with insulin implants (LinβitR) for 20 weeks significantly decreased hyperglycemia in Akita mice. Insulin treatment significantly decreased urinary albumin excretion as well as renal and urinary ACE2 activity in Akita mice. Further, insulin administration downregulated renal ACE2 and upregulated renal ACE protein expression in Akita mice. In conclusion, normalizing hyperglycemia in Akita mice with insulin decreased ACE2 protein expression and activity.

Fructose and salt rich diet from weaning to adulthood: effects on autonomic modulation in rats

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Administration of a diet rich in fructose and salt initiates a series of metabolic events resulting in hypertriglyceridemia, hypertension and glucose intolerance. The purpose of this study was to evaluate the cardiovascular and autonomic modulation induced by fructose intake during 8 weeks. Weaned (21 days old) male Wistar rats (50-60g) were divided in 4 groups and they were followed for 8 weeks: Control (CG), Fructose (FG, 10% in drinking water), Salt (SG, 1% in drinking water 10 days before the end of the protocol) and Fructose and Salt (FSG, 10% fructose and 1% salt just 10 days before the end of the protocol in drinking water). Arterial pressure (AP) and heart rate (HR) were directly recorded using a data acquisition system (Windaq, 2 KHz) in conscious and awake rats. Cardiac autonomic modulation was evaluated using pharmacological blockade (Atenolol, 8mg/Kg and Atropine, 4mg/Kg). All the experimental groups showed an increase of mean AP when compared to CG: CG=98±1,6; FG=120±3,0; SG=118±3,0 e FSG=116±2,2 mmHg). No changes in heart rate were observed, although sympathetic tonus was increased in FG and FSG when compared with CG (CG=41±7; FG=68±9; SG=51±12; FSG: 73±13 bpm) with no differences in vagal tonus. However, intrinsic HR was significantly lower in all the groups GF=384±10; S=344±8 e GFS=348±9 bpm when compared with GC (410±1 bpm). In conclusion, these data suggest that metabolic changes induced by fructose/salt rich diet during life spam in rats are accompanied by hypertension associated with sympathetic overactivity.

Poster 10

High Fat Diet Induced Downregulation of Prolyl Carboxypeptidase in ACE2 Knockout Mice

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Emerging evidence suggests that activation of the proteases, Angiotensin converting enzyme 2 (ACE2) and prolyl carboxypeptidase (PCP), are associated with metabolic pathologies. ACE2 and PCP catalyze the degradation of the vasoconstrictor Angiotensin II (ANG II) to the vasodilator ANG-(1-7). We investigated whether global loss of ACE2 affects renal expression of PCP in conditions of high fat diet induced metabolic pathologies. ACE2 knockout (ACE2KO) and wild type (WT) male mice were fed a 60% high fat (HF) or normal chow (NC) diet for 12 weeks. Using immunofluorescence microscopy and western blotting, we determined the expression pattern and abundance of ACE2 and PCP in kidney. ACE2 KO and WT mice showed no difference in glucose handling at baseline. When given a HF diet, ACE2 KO showed higher plasma glucose levels and impaired glucose tolerance compared to the WT HF and ACE2 KO NC groups. ACE2 was localized in the Bowman's capsule of the glomerulus and in the apical membrane of proximal tubules. PCP was also localized in the Bowman's capsule of the glomerulus and along the apical membrane of proximal tubules. This suggests possible co-localization of the two proteases. ACE2 was absent in kidney from ACE2KO (immunofluorescence or western). Neither the absence of ACE2 nor HF diet changed the localization of PCP. Protein intensity, normalized to ß-actin, showed no difference in PCP between untreated WT and KO (1.17 ± 0.15 vs. 1.05 ± 0.12). PCP in HF WT was significantly elevated as compared to HF KO (0.72 ± 0.06 vs. 0.44 ± 0.03, $p \leq 0.05$) while HF WT ACE2 levels remained unchanged as compared to untreated WT (0.90 ± 0.24 vs 0.94 ± 0.16). Results indicate loss of ACE2 downregulates PCP expression in metabolic pathologies. This downregulation could be explained by a possible relationship between the two proteases in metabolic pathologies.

Effects of physical training in cardiac autonomic control rats submitted to a diet rich in fructose

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The aim of this study was to evaluate cardiovascular autonomic control in trained young male rats fed a high fructose diet. Male Wistar rats (3 week age) were fed with fructose in drinking water (10%, 8wks) and randomized into sedentary (FSF,n=5) and trained (FTF, n=8), treadmill running, 5 days/week, 60 min/day, during 8 weeks) groups. Insulin resistance was evaluated by Glucose Tolerance Test was performed at week 7. Arterial pressure signals were recording using a data acquisition system. Vagal and sympathetic tonus were calculated measured by pharmacological blockade (atenolol and atropine).Statistical analysis was performed using ANOVA followed by post-hoc Dunnett. P values <0.05 were considered significant.The FT trained group had higher exercise capacity and lower insulin resistance compared to the FS sedentary group. Mean arterial pressure was similar between groups (SFS: 124 ± 6 vs. TFT: 125 ± 3 mmHg), however, the FT trained group had resting bradycardia (339 ± 10 bpm) compared to the FS sedentary group (366 ± 13bpm). The sympathetic tone was lower in the FT group (41 ± 13bpm) compared to the SFS (80 ± 6 bpm) but vagal tone was similar between groups. Our results suggest that a physical training program can be effective in attenuating the autonomic dysfunction resulting from high fructose consumption.
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Poster 12

Preconditioning by electrical stimulation (EleS) induces cytoprotective effect on cardiac stem cells (CSCs)

Kim Sun Wook, Muhammad Ashraf

Aims: Survival of stem cells following transplantation in the infarcted myocardium is a critical issue for the cell-based therapy and preconditioning (PC) of stem cells is one of the most powerful stimulus for cytoprotection. In this study, we investigated whether PC by electrical stimulation (EleS) induces cytoprotective effect on cardiac stem cells (CSCs) and determined its underlying molecular mechanisms.
Methods and Results: Sca-1^+ CSCs were isolated from male C57BL6 mice (12 weeks) hearts. PC of CSCs with EleS (^{EleS}CSCs) was carried out for 3 h at 1.5 V followed by exposure to 300 µM H_2O_2 for 5 h. Cytoprotective effects and cell adhesion ability were significantly increased by EleS as evaluated by TUNEL and lactate dehyrogenase release assay and adhesion assay. EleS increased phosphorylation of AKT, FAK and GSK3β, and decreased pro-apoptotic protein caspase-3 cleavage. Pretreatment with PI3-K inhibitor, Wortmannin and FAK inhibitor-14 abolished the pro-survival effects of EleS. Importantly, we found that *connective tissue growth factor* (*Ctgf*) was responsible for EleS-induced CSC survival and adhesion. Interstingly, *Sry* gene analysis indicated that transplantation of ^{EleS}CSCs increased their survival whereas knockdown of *Ctgf* abolished EleS-induced cytoprotective effects. Furthermore, we identified miR-378 as a potential *Ctgf* regulator in ^{EleS}CSCs by microarray and gain of function approach.
Conclusions: It is concluded that EleS enhanced CSC survival *in vitro* and *in vivo* through AKT/FAK/CTGF cascade involved in cell adhesion signaling. EleS is a promising approach for preconditioning of stem cells, and miR-378 is a novel therapeutic target for stem cell-based therapy.

THE OPERATING MECHANISM OF A NEW JAK3-FES-PLD2 SIGNALING PATHWAY THAT MEDIATES CELL GROWTH

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The enzyme phospholipase D2 (PLD2) it is central to several cellular functions, among them cell growth, which is mediated in part by a peak production of phosphatidic acid (PA) shortly after cell stimulation with growth factors. We report here the existence of a new signaling pathway that serves to regulate the phospholipase's activity: JAK3⍰Fes⍰PLD2. The two upstream kinases regulate one another and afford the cell several regulatory points. It operates very differently in normal cells (MCF10A epithelial cells) than in cancer cells (human breast cancer cell line MDA-MB-231). The reasons for an elevated PLD2 activity in cancer cells are: (1) JAK3 activity is upregulated due to EGF-R overexpression in cancer cells; (2) JAK3 activates both Fes and PLD2; (3) Fes and JAK3 phosphorylates PLD2 at an activation site (Y169/Y179), which is different than normal-cells where an inhibitory site, Y296, is phosphorylated; (4) protein-protein association between Fes and PLD2 (through the SH2 domain of FES at E469 and E472) is higher in cancer cells, which operates; and (5) PLD2's reaction product, PA, feeds back positively to Fes and activates it at a greater extent in the latter. These mechanisms provide and "overdrive" signaling pathway responsible for high proliferation in cancer cells.

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Poster 14

Apelin regulation of potassium chloride cotransport (KCC) by the nitric oxide (NO) pathway in vascular smooth muscle cells (VSMCs) and effect of osmolality changes.

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Background: Atherosclerosis-linked cardiovascular diseases (CVD) increase morbidity and mortality world-wide. Progression of atherosclerotic lesions entails recruitment of vascular smooth muscle cells (VSMCs) from the media to the intima layer of blood vessels. Morphologically and functionally distinct VSMC populations exist in physiological and pathological states. The contractile phenotype is high in healthy blood vessels, whereas atherosclerotic plaques abound in the synthetic phenotype characterized by an enhanced capacity to migrate and proliferate, thus leading to atherosclerosis. Various signaling molecules and pathways regulate such phenotypic conversion of VSMCs. NO, a signaling molecule and powerful vasodilator counteracts the effect of atherosclerosis through the NO/soluble guanylyl cyclase/protein kinase G (NO/sGC/PKG) signaling pathway in cultured VSMCs. Apelin, a recently discovered peptide that binds to a G protein-coupled receptor (APJ), plays a role in CVD. In contractile VSMCs, apelin potentiates the effect of the NO pathway and plays a fundamental role in modulating vascular function. Likewise, KCC, an electroneutral K^+ and Cl^- transport mechanism mediated by four protein isoforms in VSMCs, is also regulated by the NO pathway and is implicated in CVD. The purpose of this study is to investigate whether apelin regulates KCC through the NO pathway in contractile VSMCs.
Methods: Expression levels and distribution patterns of various contractile proteins were studied by Western blot and immunofluorescence. Rb^+, a K congener, was used to study the effect of apelin on KCC in the presence and absence of inhibitors of other K^+ transport mechanisms such as the Na/K pump (ouabain) and Na-K-2Cl cotransport (bumetanide).
Results: Findings were: 1) The identity of VSMCs was established by using markers for different structural and biological properties. 2) Both the contractile and synthetic phenotypes expressed APJ receptor. 3) PKG, a key modulator enzyme of the NO pathway, was expressed in contractile VSMCs. 4) Antibodies against the Na/K-ATPase were used as positive markers for immunofluorescence studies. 5) KCC was regulated by apelin through the NO pathway. 6) Apelin regulation of KCC was volume dependent. Studies are underway to link cellular apelin expression with transport modulation.
Conclusions: Results show that apelin modulates KCC by the NO pathway. Further studies are in progress to investigate apelin regulation of KCC at the transcriptional, translational and post-translational level. Additional information is expected to shed light on the mechanism/s of KCC regulation by apelin and their relationship to CVD.

The Flavonoid Apigenin is an inhibitor of PLD2 mediated proliferation and invasiveness in breast cancer cell line MDA-MB-231

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The MDA-MB-231 human breast cancer cell line is highly proliferative and metastatic. We report here that the invasive phenotype of this cell lines is mediated by phospholipase D2 (PLD2), under regulation of three tyrosine kinases: Janus Kinase-3 (JAK3), Feline/Fujinami poultry sarcoma (Fps) and Epidermal Growth Factor Receptor (EGFR) kinase. To dissect out the contribution of each kinase we made use of the small-molecule inhibitor, the flavonoid Apigenin. Apigenin inhibits EGFR and, consequently, PLD2-mediated cell invasion, but does not affect Fps, which is nevertheless a positive effector of PLD2. In 2-h or 16-h starved cell cultures, JAK3 switches to a PLD2-enhancing role, consistent with the needs of those cells to enter a "survival state" that relies on an increase in PLD2 activity. A multi-layered activation of PLD2 by three kinases provides regulatory flexibility and maximizes the aggressive invasive power of MDA-MB-231 cells. An additional important finding in this study is that Apigenin is a powerful inhibitor of cancer cell invasion (at concentrations of 10 nM), via PLD2, which can provide a window for potential therapeutic opportunities.

Poster 16

Selective targeting of T cell subsets with functionalized lipid nanovesicles

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T lymphocytes are essential participants of the adaptive immune system. In particular, hyperactive effector memory T lymphocytes (T_{EM}) are responsible for the cardiovascular complications of the autoimmune disease systemic lupus erythematosus (SLE). Active SLE T_{EM} cell exhibit elevated intracellular Ca^{2+} levels which contribute to the hyperactivity of this cell type. Kv1.3 channels facilitated Ca^{2+} influx in T_{EM} cells. Consequently, silencing the Kv1.3 gene in SLE T_{EM} cells can open a new specific therapeutic approach. Here we demonstrate that lipid nanoparticles (NPs) can be used to selectively target specific T_{EM} cells.
NPs were fabricated from a mixture of PC (L-α-phosphatidylcholine), PE-PEG-biotin (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[biotinyl(polyethylene glycol)-2000]) and CH (cholesterol). NPs were functionalized with either biotinylated CD3 (a T cell marker) or CD45RO (a membrane marker of T_{EM} cells) antibodies, which were bound to the vesicles via Alexa-647 conjugated streptavidin. Human peripheral blood mononuclear cells (PBMCs), human T cells, Jurkat E6-1 and MOLT-4 cell lines were used to test the specificity of NPs binding to CD3 and CD45RO of T cells.
We characterized and visualized the NPs, whose diameter ranged 100-1000nm, using dynamic light scattering method, fluorescent microscopy and SEM (scanning electron microscope). Incubation of PBMCs/Jurkat cells with antibody-coated NPs demonstrated that nanovesicles with CD3 antibody can selectively attach to T cells. Attaching CD45RO antibody to NPs we could show that MOLT-4 and primary human T cells can bind and endocyte lipid nanovsicles via the internalization of CD45RO tyrosine kinase. In our future studies we will encapsulate siRNA targeted to mRNA of Kv1.3 channel into CD45RO NPs and determine its efficiency and off-target effect on the downregulation of Kv1.3 expression in T_{EM} cells.

A Novel Approach for Localization of Gold Nanoparticles in Mice Central Nervous System

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Gold nanoparticles (GNPs) possess unique physicochemical properties that may facilitate entry into the central nervous system (CNS) where they may act therapeutically. There is little information on GNPs biodistribution in specific brain regions or extent of inflammation induction. Experiments determined the localization and neuroinflammatory response of spherical GNPs (10 nm) after IV injection in male C57Bl mice. As a supplement, a known inflammogen, lipopolysaccharide (LPS, 2 mg/kg, sc), was tested. To determine the optimal buffer concentration to maintain GNP solubility, we measured aggregation of GNPs using various PBS concentrations (10, 1, 0.1, 0.01 X). 0.01X PBS produced the least amount of GNP aggregation and was used in all studies. The next experiment verified entry of GNPs into CNS. Mice were IV injected using the tail vein (200 µg/ml 10 nm GNPs in 0.01X PBS). After 24 hrs mice were perfused transcardially with 2 % glutaraldehyde/paraformaldehyde and brains were collected. GNPs were measured using inductively coupled plasma mass spectrometry in whole brain homogenates. To specifically localize accumulation of GNPs in brain, septum, caudate, hippocampus, hypothalamus, cortex, frontal cortex, and spinal cord were dissected. Hypothalamus, hippocampus, and septum had the highest levels of GNPs (6.7, 6.2, and 4.6 µg Au/g, respectively). To evaluate brain inflammation, we used q-PCR analysis of frozen brain regions for study of pro-inflammatory mediators, LIF, CCL2 and IL1β. GNPs did not affect cytokine/chemokine expression in cortex, frontal cortex or hippocampus. LPS, as expected, caused a marked (100-fold) increase in the same cytokines. Results show that GNPs enter brain and concentrate in specific regions without eliciting an inflammatory response. Data raise the possibility of usefulness of GNPs in drug delivery and therapeutic treatment of CNS diseases.

Poster 18

Regulation of Coxsackie and Adenovirus Receptor (CAR) by Interleukin-8.

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Airway epithelia pose a formidable barrier for the entry of pathogenic viruses. The epithelial junctional proteins, which includes tight and adherens junction proteins, maintain the barrier integrity by sealing the space between the cells. Adenovirus, which causes acute respiratory infections, uses CAR as its primary receptor for entry into the host cell. CAR, an adherens junction protein, is expressed on the basolateral surface of polarized epithelia and thus is sequestered away from pathogen-exposed (apical) surface. However, despite the inaccessibility of CAR to invading adenoviruses, adenovirus infection is common. It is believed that the mechanism of entry into airway epithelia is through a structural break in the epithelial barrier that exposes the basolaterally expressed CAR to the invading pathogen. However, we have shown that an eight exon splice form of CAR (CAR^{Ex8}) can localize at the apical surface of epithelial cells and serve as adenoviral receptor. Interleukin-8 (IL-8) a proinflammatory cytokine is released by the epithelial cells and resident macrophages in the lung, during infection. We hypothesize that exposure of airway epithelia to the IL-8 increases apical adenoviral infection through increased expression and apical localization of CAR^{Ex8}. To test this hypothesis, the apical surface of polarized Calu-3 cells was treated with IL-8 for 4h and analyzed for CAR^{Ex8} expression and localization using Western blot analysis and apical surface biotinylation. Apical transduction with adenovirus carrying the β-galactosidase reporter gene was also evaluated. Our data indicate that exposure of airway epithelial cells to IL-8 increased both the expression and apical localization CAR^{Ex8}, as well as adenoviral infection. In summary, these results suggest that susceptibility to adenovirus infection in the lung might be modulated by IL-8.

Identification of Early Sepsis Biomarkers by using Dynamics in Biological Networks

Gopinath Sundaramurthy and Hamid Eghbalnia

For critically ill patients, sepsis and septic shock has been the leading cause of death. This can be attributed mainly to delayed diagnosis of the onset of sepsis. Reliable biomarkers that can detect the onset of sepsis at earlier time points could be used to improve patient outcomes. However, the underlying pathological mechanisms of sepsis and septic shock are poorly understood making the accurate and early clinical diagnosis of the disease extremely difficult. In this study, we report on a novel pathway-centric approach for early detection of sepsis through the use of time-series data. This approach uses probabilistic analysis of high-throughput data (mRNA and miRNA), along with directed and enriched signaling network information to identify functionally and differentially active pathways. Our analysis identified pathways with significantly altered interactions that were found to be associated with, for example, lymphocytes proliferation and maturation as well as cell-cell interactions. One significantly altered pathway involved the NFAT-pathway, which is to know to regulate a number of immune processes during the course of the pathology. Our integrated approach to analyzing temporal variation and enforcing consistency among multiple data sets increases the likelihood of identifying more reliable and functional biomarkers. In the future, we expect to expand this platform to aid in the discovery of prognostic and diagnostic molecular biomarkers associated with survival or other clinically relevant end-points.

Poster 20

Adenosine inhibits human T lymphocyte migration via inhibition of KCa3.1 channels

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Adenosine (Ado), a purine nucleoside that is generated by cells in response to stress and hypoxia is present at high concentrations in tumors where it contributes to the failure of immune cells to fight cancer cells. Previous studies in human lung mast cells have shown that Ado decreases their motility by inhibiting KCa3.1 channels. However, the effects of Ado on ion channels in T lymphocytes and downstream functional implications are not known. Two K^+ channels, KCa3.1 and Kv1.3, are essential to T cell function as they control Ca^{2+} signaling by regulating the driving force for Ca^{2+} influx. In this study, we tested the hypothesis that Ado asserts its immunosuppressive function in human T lymphocytes by inhibiting KCa3.1 channels. Ado inhibited KCa3.1 channels in a dose-dependent and reversible manner in activated human T cells, while no inhibition was observed of Kv1.3 channels. The action of Ado is mediated by A_{2A} and A_{2B} receptors. The inhibition of KCa3.1 channels by Ado was reversed by the selective A_{2A} receptor antagonist SCH58261 but not by the A_{2B} receptor antagonist MRS1754. The effects of Ado were mimicked by the A_{2A} receptor agonist CGS21680. Furthermore, addition of adenyl cyclase inhibitor 2'5'dideoxyadenosine and specific Protein Kinase AI (PKAI) antagonist Rp-8Br-cAMP reversed the inhibition of KCa3.1 channels by Ado. Commensurate with our previous findings that T cell migration is mediated by KCa3.1 channels, addition of either Ado or A_{2A} receptor agonist CGS21680 inhibited T cell migration, an effect mimicked by addition of KCa3.1 channel inhibitor TRAM-34. In summary, our results show that Ado inhibits KCa3.1 channels in human T cells via A_{2A} adenosine receptor and PKAI thereby resulting in decreased T cell motility. This mechanism is likely to contribute to decreased immune surveillance in solid tumors.

Tolfenamicacid inhibits collagen accumulation and expression via Sp and Smad2/3 of Transform growth factor pathway in Keloid Fibroblasts.

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Tolfenamic acid (TA), a NASID, induces apoptosis via the degradation of Specificity Proteins (Sp) in some cancer models. Keloid scar is known as fibroproliferative disorder which results in accumulation of collagen in the extracellular matrix (ECM). Thus, our aims are to test the effects of TA on inhibiting the accumulation and expression of collagen in keloid fibroblasts and to verify the mechanism of action of TA on keloid fibroblasts in vitro.
Mouse normal fibroblasts (mNFs), human normal fibroblasts (hNFs) and human Keloid fibroblasts (hKFs) were respectively obtained from mice and human dermis cell lines. Two pilot experiments were conducted to test the influence of vehicle (DMSO) and the effective concentration ($1\text{-}10^{-9}$M) of TA on mNFs. The mNFs (1×10^{5} cells/well) were treated with 0.1-100% DMSO for 3h, 48h or 7d. We found that 100% DMSO had the least effect at all exposure time periods determined by measuring cell viability and apoptosis. Then, mNFs were treated with $1\text{-}10^{-9}$ M TA to measure cell viability and apoptosis after 3h, 48h or 7d exposure respectively and with 25-200µM/mL TA+D also to measure cell viability and apoptosis after 24h, 48h or 72h exposure respectively. Our results showed that TA had a dose- and time- dependent effect on cell viability with the apoptotic rate ranging 0.6-6%. The data suggested that TA is safe to mNFs. For prospective experiments, we plan to extend our work on hNFs and hKFs. We expect that: 1) there is a least vehicle effect of 100%D at all exposure time periods; 2) selective dose- and time- dependent effect of TA on hKFs but less toxic effect on hNFs in cell viability, apoptosis, proliferation, collagen stain, collagen protein expression, and collagen mRNA expression; 3) Sp and smad3/4 are less expressed after TA treatment.

Poster 22

SONIC HEDGEHOG ACTS VIA A SMOOTHENED-DEPENDENT PATHWAY AS A MACROPHAGE CHEMOATTRACTANT

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Background: *Helicobacter pylori* (*HP*) infection leads to recruitment of monocytes/macrophages (Mø) to the stomach via a mechanism dependent upon Hedgehog signaling. Sonic hedgehog (Shh) is secreted from the parietal cell and elevated levels of the ligand are observed in circulation following acute *HP* infection. We have shown that mice with deleted Shh expression in the parietal cell and mice with a deficiency in the Hedgehog signaling protein, Smoothened, in macrophages have a deficient gastric immune response to *HP*. Furthermore, it has been shown that human peripheral blood monocytes migrate toward a Shh gradient in-vitro. Thus we hypothesize that Shh is acting as a chemoattractant via a Smoothened-dependent mechanism; macrophages lacking Smoothened will be unable to migrate towards Shh.

Methods: Bone marrow-derived macrophages (BM-Mø) were collected from control mice and transgenic mice lacking the Hedgehog signaling protein Smoothened (LysMCre/Smo^{KO}) in cells of myeloid-lineage. Gene expression in BM-Mø was analyzed by qRT-PCR. Migration of BM-Mø from control and LysMCre/Smo^{KO} was recorded overnight by confocal microscope using ibidi µ-Slide Chemotaxis chambers following loading of 1 µg/ml recombinant Shh (R&D Systems). Migration of cells was analyzed using ImageJ software.

Results: Successful deletion of Smoothened in BM-Mø derived from LysMCre/Smo^{KO} mice was confirmed by qRT-PCR. Control and Smoothened-deficient BM-Mø shared similar morphologies. A stable overnight gradient was observed in chemotaxis chambers by monitoring the diffusion of the fluorescence dye, luciferase yellow. Forward migration index, a measurement of directed movement, was significantly elevated in control BM-Mø migrating towards Shh compared to media. In contrast, Smoothened-deficient BM-Mø did not migrate towards Shh. Furthermore, velocity of cells was elevated in control cells migrating toward Shh, an effect not observed with Smoothened-deficient BM-Mø.

Conclusions: Shh acts as a macrophage chemoattractant via a Smoothened-dependent mechanism, consistent with in-vivo findings that Smoothened is required in macrophages for their recruitment to the stomach following *HP* infection.

Gastric Sonic Hedgehog Acts as a Chemoattractant for Macrophages During Tissue Regeneration

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Background: During repair Sonic Hedgehog (Shh) regulates multiple events essential for wound healing in various tissues that include cornea, heart and skin. However, the role of Shh as a regulator the immune system during gastric tissue repair remains unknown. Hypothesis: Macrophages play a central role in tissue repair. The recruitment of macrophages, that express components of the Hedgehog signaling pathway, serves as a potential mechanism through which Shh drives tissue repair. Methods: A mouse model expressing a parietal cell-specific deletion of Shh (PC-Shh^{KO}) and a separate mouse model expressing a myeloid cell-specific deletion of Hedgehog receptor Smoothened were used. Acetic acid-ulcers were induced in control, PC-Shh^{KO}, and LysMCre/Smo^{KO} mice and analyzed 1 to 7 days post-ulcer induction. Gastric samples were collected from the ulcerated and adjacent uninjured tissue, epithelium was enzymatically dissociated and macrophage recruitment was analyzed by fluorescence-activated cell sorting (FACS). Changes in cytokine, chemokine and pro-angiogenic factors were measured using a Luminex® based multiplex assay. Changes in tissue Shh concentrations were measured by ELISA. Macrophage migration was analyzed using a modified Boyden chamber. Results: In controls, ulcers healed within 7 days post-injury. Tissue regeneration was accompanied by the recruitment of $CD11b^{+}F4/80^{+}Ly6C^{high}$ macrophages to the stomach within 48 hours post injury. Control mice had elevated expression of chemokines MCP-1 and MIP-2 and pro-angiogenic factor VEGF that correlated with an increased Shh tissue concentration within 3 days post-injury. PC-Shh^{KO} mice showed complete loss of ulcer repair, the absence of macrophage recruitment and reduced Shh tissue concentrations. LysMCre/Smo^{KO} mice, in which macrophages are deficient in Hedgehog signaling, exhibited delayed repair of ulcerated tissue. in vitro macrophages migrated towards a Shh gradient. Conclusions: Gastric Shh facilitates tissue repair by acting as a macrophage chemoattractant.

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Indian Hedgehog mediates gastrin-induced proliferation in the adult stomach

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Objective: Loss of parietal cell-expressed Sonic Hedgehog (Shh) results in hypergastrinemia accompanined by increased expression of Indian Hedgehog (Ihh) and hyperproliferation of the surface mucous cells lining the pit gastric epithelium. Suppressing hypergastrinemia reverses the hyperproliferation and increased Ihh expression suggesting that these observations are largely due to elevated circulating gastrin. However, whether hypergastrinemia-induced surface mucous pit cell hyperproliferation is mediated by Ihh is unknown. Thus, we tested the hypothesis that gastrin induces gastric epithelial proliferation via activation of Ihh signaling. Method: Hypergastrinemic mice expressing a parietal cell-specific deletion of Shh (PC-Shh^{KO}) crossed with gastrin-deficient (GKO) mice (PC-Shh^{KO}/GKO) were used. Proliferation of surface pit cells was determined by BrdU incorporation. Ihh expression was quantified by laser capture microdissection (LCM) followed by qRT-PCR using tissue collected from the surface epithelium of GKO and PC-Shh^{KO}/GKO mice infused with gastrin for 7 days. Separation of epithelial-mesenchymal layers of stomachs from gastrin infused PC-Shh^{KO}/GKO mice was used to identify Ihh signaling transduction between epithelium and the underlying mesenchyme. To knock down of Hedgehog transcriptional target gene Gli1 (Gli1KD), gastric organoids developed from adult mouse stomach epithelium were transduced with Gli1shRNA lentiviral vectors. Proliferation of control and Gli1KD organoids treated with or without gastrin was assessed using EdU immunostaining. Result: Compared to controls, hypergastrinemic PC-Shh^{KO} mice had a significant expansion of surface pit epithelium indicated by increased BrdU positive cells. PC-Shh^{KO}/GKO mice did not develop hyperproliferation. GKO and PC-Shh^{KO}/GKO mice gastrin infused had increased Ihh expression and proliferation within the surface epithelium compared to the vehicle group. Gastrin-induced Ihh expression was prodominantly within the epithelium and expression of Gli1 was restricted to the mesenchyme. In vitro, Gli1KD within mouse gastric organoids caused a significant reduction of gastrin-induced proliferation. Conclusion: Gastrin-induced epithelial proliferation in the adult stomach is mediated by the Ihh signaling pathway.

Chronic Gastric Inflammation Drives the Malignant Transformation of Bone-Marrow Derived Mesenchymal Stem Cells

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BACKGROUND: Within the gastrointestinal tract, bone marrow-derived mesenchymal stem cells (BM-MSCs) contribute to healing with acute injury and promote cancer progression with chronic inflammation. In culture, BM-MSCs are inherently prone to mutation as they age. However, it is still unknown whether a similar malignant transformation occurs in vivo in response to chronic gastritis. Factors, such as transforming growth factor beta (TGFβ and Sonic Hedgehog (Shh) that are often secreted by tumors may contribute to BM-MSC transformation. HYPOTHESIS: Inflammatory cytokines and growth factors promote Shh pathway activation within BM-MSCs, inducing their aberrant proliferation and malignant transformation. METHODS: Age matched C57BL/6 (BL/6) and gastrin deficient (GKO) mice were used for isolation of BM-MSCs at 3 and 6 months of age. BM-MSC transformation was assessed by growth curve analysis, cell cycle by FACS analysis and xenograft assays. Tissue and plasma cytokine and TGFβ levels in experimental groups were analyzed by Luminex® based multiplex assay. Shh and TGFβ signaling was assessed by qRT-PCR and Western blot. RESULTS: Compared to 6 month-old BL/6 mice, GKO animals displayed atrophic gastritis and mucous cell metaplasia as a consequence of bacterial overgrowth. Both gastric tissue and circulating TGFβ and IL-6 levels were elevated in GKO mice. BM-MSCs isolated from GKO mice displayed an increased rate of proliferation and elevated phosphorylated-Smad3 indicative of active TGFβ signaling when compared to those cells isolated from BL/6 bone marrow. In xenograft assays, mice injected with BM-MSCs collected from 6 month old GKO animals displayed tumor growth. Interestingly, BM-MSCs collected from age matched BL/6 mice injected into inflamed GKO animals also developed tumor growth that was mediated by TGFβ or Shh signaling. Shh pathway activity within BM-MSCs was downstream of the TGFβ signaling. CONCLUSION: Chronic gastric inflammation induces the malignant transformation of BM-MSCs, a response that is mediated by TGFβ-induced Shh signaling.

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Site of action of the inhibitors FIPI and NOPT on PLD2

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PLD is an enzyme that catalyzes the conversion of phosphotidylcholine into phosphatidic acid. Mammals have two isoforms of PLD, PLD1 and PLD2. PLD2 has high basal activity compared to PLD1. PLD2 is highly implicated in physiological processes like chemotaxis, phagocytosis and pathological processes like cancer cell invasion. Under normal conditions activity of PLD2 is highly regulated. However many kinds of malignant cancer cells such as MDA-MB 231 cells have high levels of PLD protein as well as activity, PLD2 in specific. The highly invasive nature of these cancer cells is PLD2 dependent. One of the ways to target the highly metastatic cancer cells is via inhibiting PLD2. FIPI is well known small molecule inhibitor that inhibits PLD2 to a larger extent than PLD1. Additional small molecule inhibitors that target PLD are recently developed: NOPT, 809 and APV. Even though it is known that these inhibitors inhibit PLD2, the site of action of these inhibitors in not characterized. The goal of the present project is to determine the site of action of the inhibitors FIPI and NOPT on PLD2. In order to accomplish this, around 16 different PLD2 mutants were screened for intact lipase activity. Mutants with intact lipase activity were overexpressed in COS-7, MCF-7 and H1299 and analyzed for lipase activity in the absence or presence of FIPI or NOPT. PLD2-WT was used as the positive control. The idea is that if one or more mutants are resistant to FIPI or NOPT treatment or in other words if the lipase activity of any of the mutants is not inhibited, that mutant(s) can be further studied to confirm the site of action of FIPI or NOPT. With the preliminary studies performed we found that the PLD2 mutant L166A is resistant for NOPT treatment. However more experiments have to be done to confirm our results. Determining the mechanism of inhibitor's action on PLD2 can be of potential use in studies such as metastasis animal models where PLD2 inhibitors are used to block PLD2-mediated metastasis.

Identification of the Catalytic Site of Phospholipase D2 (PLD2) Newly Described GEF Activity

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We have demonstrated that phospholipase D2 (PLD2) is a guanine nucleotide exchange factor (GEF) for Rac2 and determined the PLD2 domains and amino acid site(s) responsible for its GEF activity. Experiments using GST fusion proteins or GST-free counterparts, purified proteins, revealed that the PX domain is sufficient to exert GEF activity similar to full length PLD2. The PLD2-GEF catalytic site is comprised of hydrophobic pocket of residues namely F107, F129, L166, and L173, all of which are in the PX domain. A nearby R172 is also important in the overall activity. In addition to the PX domain, a region in the PH domain (I^{306}-A^{310}) aids in the PX-mediated GEF activity by providing a docking site to hold Rac2 in place during catalysis. Hence PLD2 is a unique GEF with the PX being the major catalytic domain for its GEF activity, whereas the PH domain assists in the PX-mediated activity. The physiological relevance of this novel GEF in cell biology is demonstrated here in phagocytosis of leukocytes, as the GEF mutants abolished cell function. PLD2 is an enzyme that bears two activities: a GEF and a lipase. PLD2's GEF activity yields Rac-GTP and the lipase activity yields phosphatidic acid (PA). PA at low concentrations promotes GTP binding to Rac2. At high concentrations PA binds to PLD2 at PX/PH tandem, which is required for GEF activity and impedes efficient binding and interaction with its substrate, Rac2-GDP. This leads to a termination of PLD2's GEF activity. In addition we also showed that PLD2 GEF activity is regulated by phosphorylation dependent mechanisms. A tyrosine kinase, Janus Kinase (JAK3) showed negative effect on PLD2 GEF activity. Thus, this study reveals for the first time the catalytic site that forms the basis for the mechanism behind the PLD2 GEF activity and the possible regulatory mechanisms.

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The impact of FoxO1 on skeletal muscle protein synthesis

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The regulation of skeletal muscle is dependent upon the balance between protein synthesis and protein degradation. The canonical Akt/mTOR pathway plays a strong role in promoting skeletal muscle hypertrophy through upregulation of protein synthesis. The transcription factor, FoxO1, may play a role in negatively affecting protein synthesis thus leading to suppression of skeletal muscle hypertrophy. In order to study the role FoxO1 plays in regulating skeletal muscle size, a cell culture model was used in which FoxO1 estrogen receptor fusion proteins were transfected into skeletal muscle myoblasts and grown into myotubes. The myotubes were treated with 4-hydroxytamoxifen (4-OHT) to activate the FoxO1 estrogen receptor fusion proteins for 24 and 48 hours and analyzed for phosphorylated Akt (ser^{473}) and $p70^{s6k}$ (thr^{389}) via western blot. The cells were also treated with [^{3}H] phenylalanine to measure the total protein synthesis upon FoxO1overexpression. Our findings show that i) FoxO1 activation increases Akt phosphorylation compared to control cells, ii) despite amplification in Akt activity upon FoxO1 overexpression, this signaling was not transferred downstream as phosphorylation of p70s6k was blunted, iii) total protein synthesis is not impacted by overexpression of FoxO1. Despite decreased muscle size upon FoxO1 overexpression, these changes appear to be independent of alterations in the Akt/mTOR pathway and total protein synthesis. Future direction will focus on potential metabolic mechanisms that may be affected upon FoxO1 overexpression in skeletal muscle.

Ang II/Ang (1-7) balance regulates the function and gene expression of cerebral endothelial cells

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Abstract:
Brain microvascular endothelial cells (BMECs) play an important role in maintaining the health of cerebral vasculatures. In this study, we investigated the role of Angiotensin II/Angiotensin (1-7) balance in modulating the function and gene expression of BMECs. Cultured BMECs were divided into six treatment groups (n=4/group): control (basal medium), Ang II, Ang II + Losartan (AT1 receptor antagonist), Ang (1-7), Ang (1-7) + A779 (Mas receptor antagonist), Ang II + Ang (1-7). The concentration for Ang II, Ang (1-7) and A-779 was 10^{-7}mmol, For blocking AT1 receptor, Losartan (20uM) was used. BMEC function (migration and tube formation) were determined using Boyden chamber and the tube formation assay kit. The levels of endothelial nitric oxide synthase (eNOS), stromal cell-derived factor-1a (SDF-1a), CXC chemokine receptor 4 (CXCR4) and NADPH oxidase subunits (NOX2, 4) were determined by western blot and/or real-time RT-PCR. Reactive oxygen species (ROS) level was determined by dihydroethidium staining and subsequentially analyzed by flow cytometry. We found: 1) Treatment with Ang II impaired the BMEC migration and tube formation abilities, and was associated with down-regulation of eNOS and SDF-1a/CXCR4 expression and up-regulation of NOX2, 4 expression, as well as ROS level in BMECs. 2) Treatment with Ang (1-7) enhanced the BMEC migration and tube formation abilities, and was associated with up-regulation of eNOS and SDF-1a/CXCR4 expression and down-regulation of NOX2, 4 expression, as well as ROS level in BMECs. 3) Ang (1-7) could improve the compromised BMEC function and gene expression induced by Ang II. 4) These effects of Ang (1-7) and Ang II were totally abolished by A-779 and Losartan, respectively. In conclusion, Ang (1-7) counteracts Ang II in regulating BMEC function via Mas receptor and the eNOS, NOX/ROS and SDF-1a/CXCR4 pathways.

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Angiotensin Converting Enzyme 2 Priming Enhances the Function of Endothelial Progenitor Cells and their Therapeutic Efficacy

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Angiotensin converting enzyme 2 (ACE2) is a key member of the renin-angiotensin (Ang) system catalyzing Ang II into Ang (1-7). Ang II has been reported to impair endothelial progenitor cell (EPC) function and is detrimental to stroke. Here, we studied the role of ACE2 in regulating EPC function in vitro and in vivo. EPCs were cultured from human renin and angiotensinogen transgenic (R+A+) mice and their controls (R-A). In in vitro experiments, EPCs were transduced with lentivirus-ACE2 (Lenti-ACE2) or Lenti-GFP. The effects of ACE2 over-expression on EPC function and eNOS/Nox expression were determined. ACE2, eNOS and Nox inhibitors were used for pathway validation. In in vivo studies, the therapeutic efficacy of EPCs over-expressing ACE2 was determined at day 7 after ischemic stroke induced by middle cerebral artery occlusion. We found that 1) Lenti-ACE2 transduction resulted in a four-fold increase of ACE2 expression in EPCs. This was accompanied with an increase in eNOS expression and NO production, and a decrease in Nox2, 4 expression and ROS production. 2) ACE2 over-expression improved the ability of EPC migration and tube formation which was impaired in R+A+ mice. These effects were inhibited by ACE2 or eNOS inhibitor and further enhanced by Nox inhibitor. 3) Transfusion of Lenti-ACE2 primed EPCs reduced cerebral infarct volume and neurologic deficits, increased cerebral microvascular density and angiogenesis. Our data demonstrate that ACE2 improves EPC function via regulating eNOS and Nox pathways and enhances the efficacy of EPC-based therapy for ischemic stroke.

Prolonged placental activity of the hypoxia inducible gene, HIF-1a, results in changes to the placenta architecture, failure of trophoblast cells to invade and remodel the maternal arteries, premature birth, and low birth weight offspring

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Pre-eclampsia is a pregnancy-associated disorder that affects 6-8% of births in the United States. In the US alone, approximately 7 billion dollars per year is spent on the combined short and long-term effects associated with pre-eclampsia. Like many other pregnancy–associated disorders, pre-eclampsia stems from abnormal placental development. This abnormal development often results in hypertension and proteinuria for the mother, as well as premature birth and a low birth weight for the child. The placental abnormalities are typically seen within a specific cell layer, implying that they originate from a single cell lineage. Past studies have determined analogous cell types between the human and rodent placenta, allowing for the use of the rodent placenta as a model system to study the development of the human placenta. The rodent placenta consists of three cell layers: the labyrinthine, the spongiotrophoblast, and the giant cell layer. Preliminary data indicates that prolonged placental activity of the hypoxia inducible gene, HIF-1a, results in changes to the placenta architecture, failure of trophoblast cells to invade and remodel the maternal arteries, premature birth, and low birth weight offspring. The purpose of this study is to develop a lineage-specific lentiviral construct, which can subsequently be used to determine the role prolonged HIF-1a activity, in the giant cell layer, has on placental development. To determine this role, we created two constructs: a lentiviral construct consisting of the lineage-specific promoter, mPL1, linked to an altered form of HIF-1a, CA-HIF1a, and a lentiviral construct consisting of a the lineage specific promoter, mPL1, linked to the marker protein, GFP. Using the GFP linked construct, pLv-mPL1(p)-[GFP], both *in vitro* and *in vivo* studies will be used to show the lineage specific nature of the mPL1 promoter.

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Mass Spectrometry of Chelerythrine, a Protein Kinase C (PKC) Inhibitor

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Introduction: Protein kinases C occur in at least 13 isoforms and play crucial roles in cell development, proliferation and programmed cell death (apoptosis) by controlling manifold metabolic processes in the cytosol and ion and solute transport mechansisms in the plasma membrane (Steinberg 2003). Amongst the many inhibitors of these kinases, the benzo-phenanthridine alkaloid chelerythrine (CET) has attracted special attention because not only it inhibits PKC at nanomolar (Herbert 1990) but also active and passive membrane ion transport at micromolar concentrations inducing apoptosis and thus is of potential interest to cancer research. Mass spectrometry (MS) could be a tool to trace the path of and binding of CET to cytosolic and membrane proteins in human lens epithelial cells (HLEC).
Objective: To identify by MS the presence of CET in membrane transport proteins and to track down CET from its solution phase to its binding site within selected proteins of HLEC.
Methods: The compound of interest (CET) was mixed 1:1 with trifluoroacetic acid and a-cyano-4-hydoxycinnamic acid (CHCA) matrix solution, and spotted to the brushed steel target plates for MS analysis. For ionization, the target plates were placed on a Bruker Autoflex III MALDI-TOF/TOF MS and the resulting ion signals processed into a characteristic mass spectrum. HLECs exposed to a 50 µM CET solution for 30 min at 37°C, were washed and broken up with a lysing solution. Cytosol and membrane particles were separated, the latter solubilized with CHAPS to obtain a 'soluble' membrane fraction containing the transport proteins.
Results: 1) Signals for CET solubilized in acid Cl-free matrix solution yielded major peaks at molecular masses of 347 and 695 daltons, as predicted for monomeric and dimeric CET. In addition, small ion peaks with masses of 382 and 1042 daltons were found, the former likely due to CET-Cl (anion attraction at the positive iminium at position 5 of the phenanthridine ring), and the latter possibly a CET trimer involving an additional ether link. 2) Strong signals of the monomeric form lacking the anion were seen in matrix solution A diluted by 4 orders of magnitude ($7x10^{-8}$M) indicative that further dilutions will yield a CET signal, i.e. at concentration ranges to be expected for CET bound to a particulate cellular fraction. In contrast, aqueous alkaline solutions of buffer or cell fractions were generally devoid of the monomeric peak, but showed higher mass, probably dimeric CET.
Conclusions: These initial studies reveal that MALDI-TOF MS detects a "fingerprint" of the monomeric form of CET, as well as its CET-Cl salt. Presence of CET dimers are expected based on the tendency of CET to dimerize at an alkaline pH, an aspect of this research to be followed up in the future.

Assessing the effect of shear stress on Aquaporin 1 expression in vascular endothelial cells *in vitro*.

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Coronary artery bypass surgery is a procedure performed on patients suffering from coronary artery disease, using grafts of either the internal mammary artery (IMA) or the human saphenous vein (HSV). The patency rate of the HSV is less than that of the IMA due to intimal hyperplasia—smooth muscle proliferation and thickening of the blood vessel wall--that eventually increases resistance to blood flow. It is proposed that intimal hyperplasia is induced by the drastic change in environmental conditions (venous versus arterial), among which are changes in oxygen tension, shear stress, and blood flow rate. Aquaporin 1 (AQP1), a water channel protein, is expressed in the membranes of endothelial cells. The expression of AQP1 protein is increased in HSV grafts that have succumbed to intimal hyperplasia. We hypothesize that AQP1 protein abundance in endothelial cells in HSV grafts is enhanced due to changes in shear stress, making it a likely sensor in this response to environmental change. The goal of our study is to assess the effects of environmental changes on AQP1 expression in vascular cells *in vitro*. Primary cultures of arterial and venous endothelial cells will be seeded onto Ibidi µ-Slide I$^{.04}$ Luer channel slides. These slides will be attached to a bioreactor flow system and subjected to the equivalent of arterial or venous flow rate and shear stress. After 24 hours, the arterial and venous endothelial cells will be assessed for morphological changes and AQP1 expression using phalloidin staining and immunocytochemistry (ICC) respectively. We anticipate that the results of these experiments will provide insights into the reasons for HSV graft failure, and a direction for further experimentation to try to prevent it.

Poster 34

Aquaglyceroporin expression and erythrocyte omoregulation in cultures from the freeze tolerant anuran, Cope's gray tree frog, *Hyla chrysoscelis*

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Cope's Gray Tree Frog, *Hyla chrysoscelis* is a freeze tolerant anuran that accumulates glycerol during cold acclimation. We hypothesize that the osmoregulatory capabilities of erythrocytes--to tolerate changes in cell volume and to affect rapid water and solute fluxes--are likely to be most important during the events of freezing (when water leaves cells and solutes become concentrated) and thawing (when cells are exposed to a hypotonic environment when ice melts), and are dependent upon the expression and function of HC-3, an ortholog of the human aquaglyceroporin AQP3. Using erythrocytes as an *in vitro* cell culture model system, we have previously shown that HC-3 protein expression and membrane localization are dynamically regulated. The present studies explore the consequences of those changes in expression for osmoregulatory capabilities of erythrocytes. Erythrocytes cultured for 48 hrs in media made hyperosmotic (400 mOsM) through the addition of 150 mM glycerol or urea showed enhanced membrane localization of HC-3 compared with those cultured in control medium or medium made hypertonic by addition of 150 mM sorbitol or 75 mM NaCl. Cells cultured in media hypertonic with sorbitol or NaCl responded with significantly less osmotically-induced swelling (i.e. cell shape change) than erythrocytes exposed to media hyperosmotic with glycerol or urea. When aquaporins were blocked by $HgCl_2$, cells retained normal dimensions, indicating the importance of aquaporins in conferring water and solute permeability in these cells. We conclude that erythrocytes from *H. chrysoscelis* express aquaporins that confer selective permeability to glycerol and urea. We suggest that this permeability contributes to cellular tolerance of freezing and thawing. Erythrocytes from *H. chrysoscelis* provide a useful model system for studying the properties of cells with changing expression of aquaglyceroporin, HC-3.This research was supported by NSF Research Grant IOS-1121457.

Permeabilities to water and glycerol differ in erythrocytes from freeze-tolerant *Hyla chrysoscelis* and freeze-intolerant *Lithobates catesbeiana*

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Cope's gray treefrog, *Hyla chrysoscelis*, is freeze tolerant and acclimatizes to cold temperatures with an increase in plasma glycerol levels. Glycerol may act as a cryoprotectant by contributing to osmotic distribution of water between intracellular and extracellular compartments, enhancing supercooling ability, and stabilizing macromolecules. In contrast, the American bullfrog, *Lithobates catesbeiana*, is freeze intolerant and does not accumulate glycerol. Instead, *L. catesbeiana* avoids freezing by submerging in refugia that remain above freezing temperature. Thus, we predicted that because gray treefrogs accumulate glycerol and are subjected to osmotic stresses during freezing and thawing, their cells should have higher permeability to water and glycerol. We tested this prediction using erythrocytes from *H. chrysoscelis* and *L. catesbeiana.* We assessed permeability as change in shape of cells subjected to water or glycerol gradients. Erythrocytes from warm-acclimated individuals of the two species were collected in isosmotic PBS. The cell suspension was then subjected to either an osmotic gradient (PBS diluted nine-fold with MilliQ water) or a glycerol gradient (400 mOsm glycerol in MilliQ water). Control erythrocytes were preincubated in PBS with or without 0.3 mM $HgCl_2$, which blocks aquaporins that we hypothesize facilitate water and glycerol permeability. Timelapse images of RBCs were recorded using MetaMorph software on a Nikon inverted phase contrast microscope, and cell morphology was measured using ImageJ. Preliminary analyses suggest comparable water permeability between species, but lower glycerol permeability in *L. catesbeiana*. Erythrocytes from both species react to hypotonic stress induced by osmotic gradient with rapid cell shape change from elliptical to spherical. However, whereas *H. chrysoscelis* erythrocytes similarly become spherical upon incubation in glycerol solution, those from *L. catesbeiana* do not, suggesting low permeability to glycerol. The relationship between cell shape and volume is under investigation, as are the characterisitics of aquaporins found in the anuran RBCs.

Poster 36

Cell swelling induced by water and glycerol gradients in hepatocytes from warm- and cold-acclimated *Hyla chrysoscelis*, a freeze-tolerant amphibian.

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Hyla chrysoscelis tolerate freezing by minimizing damage from ice crystal formation. Freezing raises extracellular osmotic pressure, which draws water by osmosis from nearby cells. During cold-acclimation, *H. chrysoscelis* accumulate 100 mM, or more, extracellular glycerol, which may enter cells as a cryoprotectant. Hepatocytes are the likely source of glycerol synthesis and export, and also themselves must survive bouts of freezing and thawing. Therefore, we hypothesized that hepatocytes from cold-acclimated frogs would have enhanced glycerol and water permeability, facilitated by enhanced expression of aqua/glyceroporins. We tested this hypothesis by examining responses of hepatocytes to immersion in medium that imposed an osmotic gradient or a glycerol gradient. Livers of warm- and cold-acclimated *H. chrysoscelis* were perfused with perfusion media containing collagenase, and hepatocytes were dissociated in isosmotic media (400 mM for cold-acclimated animals, 300 mM for warm-acclimated animals). Hepatocytes were then subjected to hyposmotic/hypotonic challenge in water or glycerol. Shape change of individual hepatocytes, reflecting solute and water uptake, was tracked every five seconds for ten minutes after exposure to hypotonic medium. When cells were tested at 20°C, cold-acclimated hepatocytes appeared to swell and lyse more quickly than warm-acclimated hepatocytes in response to both water and glycerol challenge. These processes occurred more slowly in cells tested at ~5°C. When aquaporins were blocked with mercury (0.3 mM $HgCl_2$ for five minutes prior to osmotic challenge), hepatocytes appeared to swell slightly, but then remain unchanged through the ten minute period. These findings imply that cold-acclimated hepatocytes have enhanced membrane permeability to water and glycerol and that this change is facilitated by aqua/glyeroporins. These changes may aid in cryoprotection. The identity of the aquaporin(s) responsible for water/glycerol transport in hepatocytes is under investigation.

Membrane trafficking of aquaglyceroporin HC3 in erythrocytes from the freeze tolerant anuran, Cope's gray treefrog, *Hyla chrysoscelis*

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Cope's gray treefrog, *Hyla chrysoscelis* is a freeze-tolerant anuran that accumulates high levels of intra- and extracellular glycerol during a period of cold-acclimation in anticipation of freezing. Glycerol serves as a cryoprotectant to minimize cellular water loss and control ice crystal formation during extracellular freezing. Glycerol accumulation is thought to be facilitated by membrane proteins called aquaglyceroporins (GLPs). Previous studies have shown that the GLP HC3 is more abundantly expressed in the plasma membrane of red blood cells from cold-acclimated treefrogs as compared to warm-acclimated frogs, suggesting a functional role for HC3 in freeze tolerance. However, we do not currently know the link(s) between the environmental cues and physiological responses that lead to freeze tolerance in *H. chrysoscelis*. Therefore, the objective of our research is to explore the potential cellular and molecular mechanisms that link environmental cues (fasting, dehydration, diurnal and temperatures changes) that occur during cold acclimation, with the regulation of HC3 membrane trafficking and expression. We hypothesize that cAMP, vasotocin, and/or epinephrine participate in cell signaling pathways that result in HC3 membrane trafficking. Erythrocytes from *H. chrysoscelis* were cultured for 48 hours in complete cell culture media (CCCM) or CCCM containing glycerol. Cells cultured in CCCM were exposed to cAMP (1 uM; 30 minutes), vasopressin (10 IU; 30 minutes), or epinephrine (1 uM; 60 minutes), isolated from the culture media and fixed on microscope slides. Fluorescent immunocytochemistry was used to examine HC3 protein expression and localization. Membrane vs. cytosolic HC3 expression was quantified using Image J analysis and compared. We anticipate that the results of this study will further our understanding of the molecular mechanisms responsible for whole animal freeze tolerance. This research was supported by NSF Research Grant IOS-1121457 and the University of Dayton University Honors Program.

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Aquaporin 4 expression and distribution during osmotic brain edema and following chronic treatment of desipramine

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Blood-brain barrier water transport is facilitated by aquaporin-4 (AQP4) at astroglial endfeet. Alterations in water permeability during osmotic brain edema or following chronic treatment with antidepressant drugs may result from alterations in AQP4 localization at the astroglial endfeet. We investigated AQP4 expression and distribution in rat cerebral cortex following acute induction of osmotic brain edema or chronic treatment with desipramine. Adult male Sprague-Dawley rats were anesthetized with 1.5-2% isoflurane. To induce osmotic edema, a bolus of distilled water equivalent to 15% body weight was administered intraperitoneally. Control rats were maintained for an equivalent period of time without water injection. Animals were sacrificed 120 min later for AQP4 immunohistochemistry or measurement of cerebral water content and AQP4 expression by dot blot analysis. Data were analyzed by Dunnett's test, paired Students t-test, or Mann-Whitney U-test as appropriate and significance indicated for $p<0.05$. Mean±SEM blood serum osmolality decreased from 296±1 mOsm to 278±2 mOsm within 15 min of water injection. Cerebral water content increased from 79.8±0.2% in controls to 81.3±0.5% following water injection. Brain AQP4 expression was unaltered. Aquaporin 4 immunostaining intensity at astroglial endfeet increased from 2.6±0.04 relative intensity units (IU) in control animals to 3.2±0.21 IU in water-injected animals. Other animals were injected daily with desipramine for two weeks. They were then prepared for immunohistochemistry or measurement of brain water content as described above. Serum osmolality and cerebral water content in these animals was the same as control values. AQP4 expression was unaltered following chronic desipramine treatment; however, AQP4 immunostaining intensity increased at the astrocytic endfeet from 2.5±0.04 IU to 3.0±0.13 IU. Thus, decreased blood-brain barrier water permeability during osmotic brain edema is not a result of decreased AQP4 expression or localization at astroglial endfeet. However increased permeability following desipramine treatment may result from increased localization of AQP4 at astrocytic endfeet.

Purinergic receptors are critical for hypoosmotic volume regulation of 1321N1 human astrocytoma cells

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Brain cells swell when exposed to hypoosmotic conditions and then activate mechanisms to reestablish normal cell volume. Previous results suggest cell swelling activates ATP release which initiates volume regulation via purinergic receptors. We used genetic and pharmacologic approaches to determine whether purinergic receptors play a critical role for volume regulation of 1321N1 human astrocytoma cells. A Parental clone of 1321N1 cells which lack P2Y receptors and a second clone which stably expresses only the P2Y1 receptor (P2Y1R) was donated by Natalya Chorna (University of Puerto Rico). Methods: To measure volumes, cells were removed from the culture dish with a brief exposure to 0.25% trypsin plus 1 mM EDTA and then incubated at 37 °C in isoosmotic (290 mOsm) phosphate-buffered saline (PBS) for 30 min. Then cells were resuspended in isoosmotic or hypoosmotic (200 mOsm or 170 mOsm) PBS at 37 °C and cell volumes measured with a Coulter Counter for 30 min. ATP release was determined during the first 5 min of osmotic treatments by cloracetyaldehyde derivatization and HPLC. Results: Both clones had stable volumes in isoosmotic PBS during the 30 min experimental period. Within 3 min of resuspension in 200 mOsm or 170 mOsm PBS, Parental cells swelled to a maximal volume of 114±4% or 125±12% of their isoosmotic volume, respectively. Similar hypoosmotic-induced initial swelling was observed in P2Y1R-expressing cells. Both cell types also released ATP during exposure to 200 mOsm PBS. During the subsequent 30 min in 200 mOsm PBS, Parental cells remained swollen while P2Y1R-expressing cells recovered toward their normal volume. However, P2Y1-expressing cells failed to recover normal volume after 30 min when the P2Y1R specific antagonist, 3 µM MRS 2179 was added to the hypoosmotic PBS. Conclusion: We conclude that purinergic receptors are necessary for cellular volume regulation in this human astrocytoma cell line.

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Title: ACE2/Ang1-7 pathway protects brain from OGD-induced cell swelling and death by counteracting the Ang II/AT1 axis and in an age-dependent manner

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Abstract:
The angiotensin (Ang) converting enzyme 2 (ACE2)/Ang 1-7 and ACE/Ang II pathways are the two counteracting axes in the rennin-angiotensin system. Our previous study demonstrates that cerebral ischemic damage is enlarged in the human renin and angiotensinogen transgenic (R+A+) mice because of Ang II overproduction. This study explored whether ACE2/Ang 1-7 counteracts the effect of Ang II in cerebral ischemic injury, and whether age affects this effect. The brain slices from the young (8-10w) and aged (24-36w) R+A+ mice and triple transgenic (SARA) mice were used for the study. The SARA mice were generated by over-expressing ACE2 in neurons of R+A+ mice. Oxygen and glucose deprivation (OGD) was introduced to brain slices for mimicking ischemic stroke. For some experiments, the AT1 receptor blocker losartan (20μM) and angiotensin1-7 (10μM) were added in the perfusion buffer during OGD. Brain tissue swelling was determined by measuring intrinsic optical signal (IOS, light transmission through the slice) as an index. The number of cell death was determined by counting the propidium iodide (PI)-positive cells under confocal microscope. The severity of cell injury was analyzed by measuring the level of LDH in the perfusion buffer by using a commercial kit. Results showed: 1) OGD increased IOS, PI-positive cells and LDH release were more in the brain slices of R+A+ mice than in the SARA mice; 2) Treatment with angiotensin 1-7 reduced OGD-induced increase of IOS in the slices of young or aged R+A+ and SARA mice. 3) Losartan reduced more IOS value in aged SARA mice than in young SARA mice. Our data indicate that ACE2/Ang1-7 pathway protects the neuron from ischemic injury and this effect is in an age-dependent manner.

Characterization of stimulation-induced volume changes in the Ca1 region of rat hippocampus slices

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During hypoosmotic cell swelling, brain cells activate volume-regulated anion channels (VRAC) to release taurine for volume regulation. Brain cells also swell during neuronal activity; however, mechanisms of cell swelling and volume regulation during functional brain activity are unclear. To examine these processes, volume changes in the Ca1 region of 400 µm rat hippocampal slices were evaluated during electrical stimulation of the Schaffer collateral pathway. Field potentials in Ca1 were monitored to assure viability of the slice and to evaluate synaptic transmission. Slices were perfused with artificial cerebrospinal fluid (aCSF) and irradiated with white light while images were acquired to measure light transmission through Ca1 (intrinsic optical signal or IOS)as an indirect measure of cell volume. Low Ca^{2+} aCSF was used to block synaptic transmission. The influence of VRAC and AMPA receptors was examined using VRAC inhibitors, 100 µM niflumic acid or 20 µM DCPIB, and the AMPA receptor antagonist, 25 µM CNQX. Data were analyzed using ANOVA with Dunnett's *post hoc* test ($p<0.05$ indicated significance). Changes in IOS were dependent on stimulation frequency between 1 Hz and 10 Hz. Stimulation at 5 Hz or 10 Hz for 5 min increased IOS by 1.68±1.21% or 2.52±1.40%, respectively. A significant recovery in IOS was seen following stimulation at 5 and 10 Hz. Synaptically blocked slices experienced a similar increase in IOS during stimulation, but did not recover fully. Niflumic acid had no significant effect on IOS, but DCPIB showed a significantly slower rate of recovery possibly from inhibition of glutamate transport. Slices exposed to CNQX swelled similar to controls; however, the initial rate of IOS recovery was increased. The findings suggest that synaptic activity has little effect on swelling but is required for volume recovery. Glutamate accumulation may be important for swelling, but volume recovery is not dependent on VRAC.

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Evaluation of G forces in Helmet Collision and Concussion Risk

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In contact sports, when two players run towards each other from opposite directions and collide, at the point of collision the momentum is transferred. A force develops through the skin of the helmet, liner, skull to the brain based on a series of mechanical events. This process evokes a certain amount of G-forces on the skull which is transferred to the brain causing it to rotate, and may lead to indications of a concussion. The objective of this research is to understand the collision events via helmet to the brain. Data obtained from literature and National Football League website were evaluated to determine G-forces developed and transferred from the helmet to the brain in professional athletes.-Monte Carlo simulations were used and data was fitted with normal and weibull distributions for a specific risk of injury when a specific G-force was generated. Accordingly, a 10%, 50% and 90% risk levels were assigned and mathematical equations developed to predict such risks. Concussion collision risk in children 10-15 and 15-18 to college were also analyzed. Although little understood, the analysis this research conducts from children-adults and 1 type of helmet and padding provides the awareness of how important it is to take concussions, whether severe or mild, very seriously.

Membrane trafficking produces distinct β-adrenergic signaling for Cl^- secretion and K^+ secretion in guinea pig distal colonic mucosa

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Epinephrine (epi) activated Cl^- and K^+ secretion in isolated mucosa from guinea pig distal colon, measured as short-circuit current. These components of the response were distinguished by inhibition of Cl^- secretion with a β2 antagonist (ICI-118551) that spared K^+ secretion; propranolol inhibited both. Interfering with endocytosis prolonged the transient Cl^- secretion and inhibited sustained K^+ secretion. Both the dynamin inhibitor dynasore and the clathrin-coated-pit blocker mono-dansylcadaverine produced this response. Dynasore also inhibited the sustained epi-induced increase in mucosal cAMP, while leaving the transient cAMP increase intact. Manipulating the ubiquitylation cycle by inhibiting ubiquitin-E1 ligase (UBEI-41, BioGenova) prolonged the epi-induced Cl^- secretory transient and slowed the onset of K^+ secretion. Conversely, inhibiting de-ubiquitylases (PR-619, LifeSensors) alone stimulated K^+ secretion, while blunting Cl^- secretion and producing a non-additive increase in K^+ secretion with epinephrine. Disruption of the actin (latrunculin B, cytochalasin D) and microtubule (nocodazole) cytoskeleton inhibited epi-induced K^+ secretion without altering Cl^- secretion. Together these results support an activation process involving internalization of β2-adrenergic receptors that terminates signaling for Cl^- secretion, and β-adrenergic stimulation of K^+ secretion that requires receptor internalization prior to activation of the sustained cAMP signaling cascades. [NIH DK65845]

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Changes in ion transport status influence *Bacteroides thetaiotaomicron* growth

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Microbial dysbiosis plays a key role in complex diseases such as Inflammatory Bowel Diseasse (IBD), diabetes, obesity and *Clostridium-difficile* associated disease. A better understanding of how the gut microbiota are assembled and maintained is critical to the treatment of these complex chronic diseases. Na^+/H^+ exchanger isoform 3 (NHE3) plays an integral role in intestinal Na^+ absorption and $NHE3^{-/-}$ mice exhibit chronic diarrhea. Quantitative PCR (qRT-PCR) using 16S ribosomal DNA bacterial primers revealed that $NHE3^{-/-}$ mice exhibit microbial dysbiosis in both the lumen and mucosa-associated bacteria with regional decreased *Firmicutes* and increased *Bacteriodetes.* The genus *Bacteroides* and specifically the species *B. thetaiotaomicron (B. theta)* were increased only in the $NHE3^{-/-}$ ileum. This correlated with increased *fut2* mRNA and fucosylation in the $NHE3^{-/-}$ ileum. The $NHE3^{-/-}$ ileum was found to contain 42.8mM Na^+ and this concentration was found to be optimal for *B. theta* growth *in vitro*. Replacement of Na^+ for other cations *in vitro* further showed *B. theta* growth was reliant on Na^+ concentration. These data together show a possible mechanism for *B. theta* proliferation and contribution to microbial dysbiosis in the $NHE3^{-/-}$ mouse intestine.

Mechanisms Regulating the Hippocampal Taurine Transporter Protein in Rat Brain.

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In cytotoxic edema, net efflux of taurine from neurons and accumulation by astrocytes contributes to neuronal volume regulation and astrocytic swelling. Taurine is accumulated in both cell types by a sodium- and chloride-dependent 72-75 kDa protein transporter, TauT. TauT functional activity decreases in osmotically swollen neurons but is unaltered in swollen astrocytes, *in vitro*. This swelling-induced downregulation of neuronal TauT activity is blocked with the tyrosine kinase (TK) inhibitor, genistein. In contrast, PKC activation has no effect on neuronal TauT, but inhibits astrocytic TauT. Thus, we hypothesize that neuronal TauT activity is regulated by a TK signaling pathway whereas astroglial TauT activity is regulated by serine/threonine kinases. This differential regulation contributes to neuronal volume regulation and astrocytic swelling via taurine redistribution during cytotoxic brain edema. Primary neuronal and astrocytic cultures from rat hippocampus were incubated under iso- or hypo-osmotic conditions in the presence or absence of activators or inhibitors of TK or PKC. Subcellular TauT localization was measured after 30 min using cell surface biotinylation and western blot analyses. Phosphorylation was measured after 30 min using immunoprecipitation and western blot analyses with phosphoprotein-specific antibodies. Neuronal and astroglial TauT was found on the plasma membrane in isoosmotic conditions. However, cell surface biotinylation of TauT decreased in swollen neurons while phosphorylation of tyrosine residues increased. Surface biotinylation and phosphorylation of TauT in astrocytes were unaffected by cell swelling or treatment with 1 μM PMA. The results suggest the signal for neuronal TauT translocation from the cell membrane during hypoosmotic cell swelling involves tyrosine phosphorylation. Membrane localization of astroglial TauT was unchanged during hypoosmotic cell swelling or PKC activation under isoosmotic conditions. These changes may account for the observed reduction in functional TauT activity in swollen neurons and may contribute to neuronal volume regulation during cytotoxic edema.

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Novel role for divalent metal-ion transporter-1 in the absorption of iron derived from heme

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Iron deficiency is the most prevalent micronutrient deficiency worldwide. Whereas dietary heme iron offers greater bioavailability than nonheme iron, relatively little is known about the mechanisms of heme-iron absorption. Heme (ferrous protoporphyrin IX) is thought to be taken up intact into the enterocyte via a receptor-mediated endocytosis. How ferrous iron (Fe^{2+}) is exported from the endosome (or lysosome) after its liberation from heme is not known. Divalent metal-ion transporter-1 (DMT1) is a widely expressed Fe^{2+} transporter that serves the uptake of nonheme iron at the intestinal brush border. We considered a role for DMT1 also in the endo-/lysosomal export of heme-derived iron, in analogy with its established role in endosomal transport of iron derived from transferrin. We tested a role for DMT1 in intestinal heme-iron absorption by examining the intestinal handling of heme in a mouse model lacking intestinal DMT1 (i.e. $DMT1^{int/int}$). Generation of the $DMT1^{int/int}$ model, by crossing floxed DMT1 and villin-Cre transgenic lines, was described previously [Gunshin et al (2005) *J. Clin. Invest.* 115, 1258–1266]. The $DMT1^{int/int}$ mouse exhibited a severe hypochromic–microcytic anemia—characterized by profound decreases in hematocrit at age ≈ 120 d (Hct, $DMT1^{int/int}$, 7% ± SD 2% cf. wildtype, 44% ± 4%; $n = 7–9$), hemoglobin concentration, mean corpuscular volume, and serum iron—accompanied by cardiac hypertrophy, splenomegaly, and severely depleted nonheme iron stores (liver, spleen). Bypassing the intestinal lesion by administering intraperitoneal iron injections (12.5 mg Fe at 28 d and 56 d) corrected the iron-deficiency anemia phenotype of the $DMT1^{int/int}$ mouse. Feeding wildtype mice with red blood cells (washed, packed cells resuspended in an equal volume of saline, 0.2 ml/10 g body weight, weekly for 6 weeks) via intragastric gavage increased their nonheme liver iron stores by over 130%, confirming a functional heme-absorptive pathway in wildtype animals. Feeding red blood cells to $DMT1^{int/int}$ mice had no effect on hematological variables (e.g. Hct, 10% ± 3%; $n = 4$), blood-iron variables, or liver nonheme iron content. Our data reveal a novel role for DMT1 in heme-iron absorption. Future studies will be directed at measuring [^{59}Fe]heme absorption by direct methods and localizing DMT1 within the heme-absorptive pathway. Acknowledgments: PHS Grants DK080047, DK078392, and DK060444

Secretagogue Modulation of Acid Secretion in the Guinea Pig Distal Colon

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The guinea pig distal colon secretes acid as well as Cl^- and K^+. Epinephrine (epi), prostaglandin E2 (PGE2), and carbachol (CCh) stimulate the secretion of Cl^- and K^+ into the colonic lumen. Previous pH stat studies indicate a ouabain sensitive transepithelial acid secretion of 1.0 $\mu eq \cdot cm^{-2} \cdot h^{-1}$ due to a colonic H,K-ATPase [Suzuki, APS253:G155,1987]. Experiments from the current study without bicarbonate indicate an average ouabain sensitive rate of 1.3 $\mu eq \cdot cm^{-2} \cdot h^{-1}$. All previous pH stat experiments exclude bicarbonate. Further experiments were conducted using a physiological bicarbonate and carbon dioxide buffer. Experimental protocol in bicarbonate conditions involved two tissues set up in Ussing chambers under pH stat control. Application of mucosal ouabain to one tissue resulted in net bicarbonate secretion, while the other tissue remained ouabain free and resulted in net acid secretion. The difference between the rate of alkalization and the rate of acidification of the two tissues represented the ouabain sensitive acid secretion. The presence of bicarbonate in the serosal solution showed a ouabain sensitive rate of approximately 1.5 $\mu eq \cdot cm^{-2} \cdot h^{-1}$. Secretagogue stimulation experiments indicated little response to the K^+ stimulant epi. However, the Cl^- stimulators PGE2 and CCh resulted in a spike in ouabain sensitive acid secretion of approximately 0.3 $\mu eq \cdot cm^{-2} \cdot h^{-1}$. These results demonstrate that activation of Cl^- secretion is accompanied by acid secretion.

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The apical NKCC1 cotransporter debate

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Choroid plexus epithelial cells (CPECs) secrete cerebrospinal fluid (CSF) and regulate its electrolyte composition. CPECs express Na^+/K^+ ATPase and Na^+-K^+-$2Cl^-$ -cotransporter 1 (NKCC1) on their apical membrane (CSF-facing), deviating from typical basolateral membrane location in secretory epithelia. Given this noncanonical location of NKCC1 for a secretory epithelial cell, the direction of net ion fluxes mediated by this cotransporter and associated water fluxes, under physiological conditions, is controversial in CPECs. NKCC1-mediated ion and water fluxes are tightly linked, thus their direction can be inferred by measuring cell volume changes in single cells following NKCC1 inactivation. Hypotheses: under physiological conditions NKCC1 is working in the uptake mode and maintains normal cell water volume in CPECs, hence genetic ablation of NKCC1 will produce cell shrinkage due to unbalanced net efflux of solutes and water. Electron microscopy revealed NKCC1-/- CPECs are severely shrunken, forming large dilations of their basolateral extracellular spaces, yet remain attached by apical tight junctions. Differential interference contrast microscopy measurements confirmed that freshly dissociated CPECs from NKCC1-/- mice are half the size of CPECs from WT ($p < 0.01$). These results strongly suggest that under physiological conditions NKCC1 is constitutively active, works in the uptake mode, and is necessary to maintain normal cell water volume.

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Rb Influx in K-Cl Cotransporter 3 (KCC3)-transfected Human Embryonic Kidney 293 (HEK293) Cells and Effect of External Na

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Background: K-Cl cotransport (KCC) electroneutrally extrudes K and Cl under physiological conditions. KCC is encoded by 4 genes (SLC12A4-7) with at least four major protein isoforms (KCC1-KCC4). Major functions of KCC involve cell volume and Cl regulation through several physiologically relevant signaling pathways, whereas abnormalities in its structure/function relationships are associated with numerous pathological conditions. Cells from most if not all tissues express more than one protein isoform albeit in different proportions. Thus, KCC function is defined by the net interplay between the expressed isoforms. The purpose of this study is to characterize in a human cell line, the functional properties of one of the protein isoforms (KCC3), which has been linked to several pathologies.
Methods: HEK293 cells transfected with KCC3 served as a model to study KCC activity. Rb, a K congener, was used to study KCC in the presence and absence of ouabain or bumetanide, inhibitors of other K transport mechanisms (Na/K pump and Na-K-2Cl cotransport (NKCC), respectively) and in Cl or Cl-free medium (sulfamate replacement). The activities of KCC and NKCC, which are reciprocally modulated by most activators and inhibitors, and that of the Na/K pump were measured under the same experimental conditions to assess the behavior of KCC3-transfected HEK293 cells. The effect of Na-free medium (N-methyl-D-glucamine replacement) was also tested.
Results: Main findings: 1) Rb uptake in KCC3-transfected HEK293 cells was similar to that reported for non-transfected cells from different tissues under basal conditions, and was linear up to 10 min. 2) Likewise, NKCC activity was similar to non-transfected cells although Rb uptake through this system was not linear even at the earliest time point tested (2.5 min). 3) NKCC was more than 3 fold higher than KCC, as reported for non-transfected cells from various tissues. 4) When the Na/K pump, NKCC and KCC activities were measured simultaneously, NKCC was the largest component (25 %, 63 %, and 11 %, respectively, of the total Rb flux) in agreement with previous findings in several non-transfected cell types. In addition, KCC was about 6 fold higher than NKCC in Na-free medium.
Conclusions: KCC3-transfected HEK293 cells behave like non-transfected cells for KCC, NKCC and Na/K pump activity under baseline conditions or in Na-free medium and thus constitute a promising mammalian experimental model to study the specific role of KCC3 in cell volume regulation and in different signaling pathways, a much awaited outcome elusive until now for mammalian cells.

Poster 50

Magnesium sensitivity of TRPM7 channels in leukocytes

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TRPM7 channels are highly expressed in T lymphocytes and other white blood cells. Unlike other TRP family members, TRPM7 (and the closely related TRPM6) have dual ion channel and protein serine/threonine kinase activities. The kinase domain of TRPM7 belongs to the atypical kinase family and its role in the regulation of channel activity is not well understood. Both native and heterologously expressed TRPM7 have been shown to be sensitive to cytoplasmic free Mg^{2+} with an IC50 of ~600 uM. We performed patch clamp electrophysiology experiments using whole-cell and cell-free configurations in order to examine the Mg^{2+} inhibition in detail. Surprisingly, we find that in whole cell, Mg^{2+} inhibits through two sites of high (10 uM) and low (165 uM) affinity. Using the inside-out patch recording configuration we constructed a Mg^{2+} dose-response relation for TRPM7 channels which showed an overall similarity to our results in whole-cell. The primary effect of Mg^{2+} at the single channel level was a dose-dependent reduction in the *number* of conducting channels. We did not observe intermediate low open probability states in the presence of Mg^{2+}. The mechanism of channel inhibition, therefore, must involve an extremely steep reduction in the open probability leading to channel closings. An additional effect of Mg^{2+} at high concentrations was a monotonic drop in unitary channel conductance by approximately 20%. Mg^{2+} effects were reversible upon washout. Interestingly, the potency of Mg^{2+} grew upon multiple applications of the same concentration, demonstrating sensitization. Additionally, we report that monovalent hTRPM7 possesses two Mg^{2+}-sensitive conductance states of 39.0 pS and 18.6 pS.

The synaptic vesicle protein, Rab3A, is required for homeostatic synaptic plasticity in mouse cortical neurons

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The nervous system is faced with perturbations in activity levels throughout development and in disease or injury states. Neurons need to be plastic to adapt to these changes in activity, but also need to maintain circuit firing within a normal range to stabilize the network from becoming too excited or too depressed. Homeostatic synaptic plasticity, the compensatory increase or decrease in synaptic strength as a result of excessive circuit inhibition or excitation, is a mechanism that the nervous system utilizes to keep network activity at normal levels. Rab3A is a small GTPase that binds synaptic vesicles by switching between its active GTP-bound form and its inactive GDP-bound form. Previously, we have shown at the mouse neuromuscular junction (NMJ) that a mutated form of Rab3A, Rab3A *earlybird* (*ebd*), abolishes the compensatory miniature excitatory postsynaptic current (mEPSC) amplitude increase in response to activity blockade by tetrodotoxin (TTX), while at the Rab3A$^{-/-}$ NMJ the effect of TTX is blunted (Wang et al., 2011). At the NMJ, the increase in mEPSC amplitude after activity blockade is not accompanied by an increase in postsynaptic receptor levels. These results demonstrated that normally functioning Rab3A is required for homeostatic synaptic plasticity at the NMJ, possibly through a presynaptic mechanism. Here, we examined whether Rab3A is also necessary for modulating homeostatic synaptic plasticity at central synapses, by globally blocking network firing in dissociated mouse cortical neuron cultures via a two day TTX treatment. We used whole-cell voltage clamp to record miniature excitatory postsynaptic currents (mEPSCs) from control and TTX-treated cells of wild-type (WT), Rab3A$^{-/-}$, and Rab3A$^{ebd/ebd}$ pyramidal neurons at 13-14 days in vitro, and found that in both Rab3A$^{-/-}$ and Rab3A$^{ebd/ebd}$ cultures, the increase in mEPSC amplitude was prevented. These results are surprising because it is predominantly believed that homeostatic synaptic plasticity at central synapses is due to modifications in postsynaptic receptor levels, but our results here and in our previous studies at the mouse NMJ suggest that a presynaptic mechanism is also involved.

Poster 52

Can an Inhibitory synaptic potential accelerate neuronal firing rate?

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The regulation of motoneuron (MN) firing rate is one of several proposed functions of recurrent inhibition, and this regulation certainly depends on the after-hyperpolarization that follows action potentials. The present study was performed to examine the interaction between AHP and recurrent inhibitory post synaptic potentials (RIPSPs) in the adult rat in vivo. Intracellular recordings were obtained from lumbar MNs in rats anesthetized by isoflurane and acutely deafferented. Repetitive firing was induced by suprathreshold intracellular current injection. Recurrent inhibition was activated antidromically at different frequencies (2Hz, 20Hz, and 30Hz). Analysis of inter-spike intervals was compared based on the timing of RIPSP occurrence. RIPSP coincidence with AHP resulted in shorter inter-spike intervals in all trials, in 6 MNs. The increased firing observed was correlated with the RIPSP amplitude which was correlated with stimulation frequency, being larger at 20Hz. On the other hand, RIPSP coincidence with the action potential decreased the size or delayed the occurrence of the spike consistent with earlier literature. Thus, recurrent inhibition decreased or increased MN firing rate depending on its timing relative to action potentials in the train. Our findings also suggest that the frequency dependence of RIPSP amplitude promotes repetitive firing of MNs at preferred rates.

Adrenergic agonists do not modify the *in vivo* cholinergic eccrine sweating dose-response relations

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Muscarinic and both α- and β-adrenergic receptors have been identified on secretory clear cells of human eccrine sweat glands. The potential interactions of these receptor populations in an intact *in vivo* model are unclear. To probe for sub-additive, additive, or synergist relations of multiple agonists, 15 healthy subjects had 6 intradermal microdialysis placed in dorsal forearm skin perfused with: 1 & 2) 6 log M doses of acetylcholine (ACh); 3 & 4) Ach doses plus 200 or 80 µM isoproterenol; 5) ACh doses plus 3.16 µM norepinephrine and 1 mM propranolol; 6) Ach doses plus 100 µM phenylephrine. Sweat rate was measured via capacitance hygrometry and dose-response relations were assessed by logistic modeling to determine the dose causing ½ of maximal response (ED50). Additive or synergist relations would result in leftward shift in the dose-response curve and sub-additive relations in a rightward shift. ACh only produced dose-response relations with ED50 of -1.2±0.2. The addition of a β-adrenergic agonist did not significantly alter the ED50 (200 µM = -1.3±0.1 and 80 µM = -1.1±0.3). Neither the addition of an α1- adrenergic agonist nor α1- and α2-adrenergic agonists co-perfused with a β-adrenergic antagonist significantly affect ED50 (-1.2±0.1 and -1.4±0.1, respectively). Combined, these data indicate that adrenergic agonists, used in concentrations that work independently, do not modify the *in vivo* cholinergic eccrine sweating dose-response relations. These results do not provide evidence for multiple agonist combinations causing augmented sweating responses as are seen in clinical conditions such as focal hyperhidrosis.

Poster 54

Reduced motoneuron excitability contributes to intensive care unit acquired weakness

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Background: Despite years of study, the mechanisms underlying intensive care unit acquired weakness (ICUAW) remain unclear. Studies to date suggest that myopathy and neuropathy are the primary causes of weakness. However, we noticed that in some patients neither myopathy nor neuropathy seemed sufficient to account for severe weakness. We set out to identify the additional contributor to weakness in these patients.

Findings: In patients with ICUAW following sepsis, motor unit recruitment was limited to few motor units, which fired at low rates. The reduction in motor unit recruitment could not be accounted for by neuropathy, suggesting that functional impairment within the central nervous system (CNS) contributes to ICUAW. To specify the contribution of CNS dysfunction to reduced motor unit recruitment, we studied the intra-spinal portions of motoneurons to determine their capacity for initiating and repetitively firing action potentials in a rat model of sepsis. Studies of single action potentials and passive membrane properties of motoneurons from septic rats were all normal, suggesting excitability was normal. However, motoneurons exhibited striking abnormality and dysfunction during repetitive firing. The sustained firing that underlies normal motor unit activity and smooth force generation was slower, more erratic, and often intermittent in septic rats.

Interpretation: Our data are the first to suggest that reduced excitability of neurons within the CNS contributes to ICUAW. This represents a paradigm shift for a field that is focused on neuropathy and myopathy as the primary sources of weakness following critical illness. The mechanism underlying reduced excitability of motoneurons appears to be novel and distinct from the sodium channelopathy we previously identified as underlying reduced excitability of muscle and peripheral nerve. We propose that restoration of excitability of neurons within the CNS should be a goal for development of therapy for ICUAW.

The Comparative Effects of Entertaining versus Educational Stimuli on Recall and Brainwave Activity

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Most people recall information best when it pertains to a subject that has been presented in an entertaining or engaging manner. In this study, an electroencephalogram (EEG) was used to obtain delta and beta brain wave patterns from 15 female participants as they watched three videos on male reproduction with as dry, engaging, or entertaining presentation styles. These waves in particular were chosen because they give an indication of motivation and concentration on a specific task, respectively. The Biopac EEG was used to observe the participants' brainwaves while they watched these randomly ordered videos and answered five questions about each video for a total of 15 questions. It was hypothesized that the beta waves would show a higher frequency and amplitude when participants viewed the dry video and recalled its information compared to viewing and recalling information from the other videos, and lower frequency and amplitude when analyzing the delta waves. The results from the procedure were analyzed with paired sample t-tests. No significant differences could be found when comparing the waves neither between the dry, engaging, or entertaining videos nor between the dry, engaging, or entertaining questions ($p \geq .05$). However, when comparing the acquisition of information during the video to the recall during questioning, both beta and delta wave amplitudes from the dry video condition and the questions pertaining to the video results were observed to be significant ($t(14) = -2.24$, $p = 0.042$ and $t(14) = -2.35$, $p = 0.034$, respectively). This study found a significant difference in the beta and delta waves between acquisition and recall, but did not find differences between conditions during recall. Future studies should increase the sample size and expand the variety of videos watched and to establish if presentation style does indeed impact memory acquisition.

Poster 56

The Effects on Reaction Time When Introducing Distracting Stimuli

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The relationship between distraction and reaction time is exemplified by the prevalence of multitasking; activities such as texting or talking on the phone while driving can have dangerous consequences and several states have outlawed driving while texting. This experiment sought to find a relationship between a distracting stimulus and reaction time. It was hypothesized that adding a distracting stimulus would increase reaction time. Fourteen participants were asked to respond to a tone sounding at random intervals by pressing a button generated by the Biopac system. In the control, all participants closed their eyes and pressed a button when they heard a sound. In the second trial, participants silently read a passage while responding to a randomly generated tone. The final trial required the participants to read a different passage aloud while pressing a button in response to the randomly generated tone. The gathered response times in each trial indicated that reading silently significantly increased the reaction time of the participants when compared with control ($t(13)=-3.64$; $p=0.003$), and reading aloud also significantly increased reaction times of participants when compared with control ($t(13)=-5.89$; $p<0.001$). These results reflected the hypothesis that distracting stimuli increased reaction times. This research exhibits how multitasking culminates in divided attention and deficiency in motoric tasks. Future research could add a distraction to a simulated driving scenario to further validate these findings.

Use of DTI for Quantification and Analysis in Neuroimaging Data

Kevin Hatcher

In the work the use of (DTI) Diffusion Tensor Imaging will be used for the quantification and analysis of neuroimaging data. The data is obtained from 30 participants (15 controls and 15 patients) who were exposed to General Anesthetics during the early infancy stages of life. The exposure is assumed to cause white matter structure changes through human development. The significance was to see if there is any volumetric differences between patients and controls at the two regions of interest (ROI) the basal ganglia and prefrontal cortex.
DTI is the specialized form of Magnetic Resonance Imaging (MRI) that allows the user to view changes in white matter tract fibers. ROIs were quantified using Diffusion ToolKit and TrackVis (http://trackvis.org/). Raw DTI data were input into Diffusion Toolkit and tractography data were created and displayed with TrackVis, where ROIs were segmented manually

Poster 58

Use of the Cam Kinase Kinase β inhibitor STO609 is precluded in Ca^{2+} imaging studies

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Evidence suggests that the energy-sensing enzyme AMP-activated protein kinase (AMPK) may play a critical role in oxygen-sensing by the carotid bodies. To activate AMPK, it must be phosphorylated at Thr-172 (alpha subunit) by upstream kinases. To date, these kinases have been identified as LKB1, CamKKβ, and TAK1. This study's objective was to determine the importance of CamKKβ in the hypoxic chemotransduction mechanism of oxygen-sensing Type I cells within the mouse carotid body.

Type I cells were loaded with Fura-2 (5 µM). Intracellular Ca^{2+} was measured by exposing cells to 340nm and 380nm light and recording emission fluorescence at 510nm. Initial experiments were to see if Ca^{2+} increase during hypoxic exposure (10 Torr) could be altered by the CamKKβ inhibitor STO609 (100 µM). However, STO609 is green in solution and application caused large emission signal deviations. Faster changes in the 380nm signal caused a 340/380 ratio fall, suggesting intracellular Ca^{2+} decreased. Importantly, in the absence of cells, STO609 increased 510nm emission, indicating the inhibitor itself was fluorescing.

To avoid this artifact, Type I cells were loaded with a 'red emission' Ca^{2+} indicator dye, x-Rhod-1 (5 µM), exposed to 580nm light, and emission was recorded at 602nm. Cells were excited with high K^{+} (50mM). During elevated Ca^{2+}, STO609 (100 µM) was applied and an immediate fall in the emission signal was observed. Additional experiments in unstimulated cells showed a similar fall in emission signal.

This immediate effect of STO609 is unusual as incubation >10min is usually required to inhibit CamKKβ. Therefore, the fall in x-Rhod-1 emission is also likely artifactual. To determine if STO609 directly interferes with x-Rhod-1 emission, future experiments will use 0mM Ca^{2+} solutions containing ionomycin.

Presently, these data preclude STO609 use in Ca^{2+} imaging experiments.

Obese Levels of Leptin Initiate Calcium Signaling Events in Rat Carotid Body Type I Cells

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Levels of the energy-signaling hormone leptin are elevated in obese individuals and it has been established that leptin is a respiratory stimulant (Malli et al, 2010). Recent evidence suggests that leptin receptors are present on the Type I cells of the carotid bodies, organs that are critical to the control of breathing (Porzionato et al, 2011). However, to date, there is no evidence that activation of leptin receptors could alter the excitability of the carotid bodies and therefore influence breathing by this mechanism.

Obese levels of leptin (100 ng ml^{-1}, 10 minute exposure) evoked calcium signaling responses in 73 % of Type I cells (n = 11). Responding cells significantly increased their Fura 2 F_{340}/F_{380} ratio by 0.12 ± 0.03 ratio units (n = 8, $p < 0.03$). Removal of extracellular Ca^{2+} did not significantly change resting ratio levels compared with controls but abolished Ca^{2+} signaling events in response to leptin (n = 4).

These preliminary data indicate that acute application of leptin to isolated Type I cells evokes small Ca^{2+} signaling events. These Ca^{2+} signals are mediated by an as yet undetermined calcium entry pathway. Future work will focus on characterizing the Ca^{2+} entry pathways activated by acute leptin and will also investigate changes in leptin signaling and carotid body excitability in obese animals with chronically elevated leptin. Importantly, these data represent the first evidence that leptin receptors are functional in the carotid body and that their activation or desensitization could influence Type I cell excitability and therefore breathing.

Malli et al, (2010) *Respir Res*, 11: 152
Porzionato et al, (2011) *Brain Res* 1385: 56-67

Poster 60

Chemosensitive signaling of locus coeruleus neurons in the Bullfrog, *Lithobates catesbeianus*

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The locus coeruleus (LC) senses changes in CO_2/pH and influences breathing patterns to control acid-base homeostasis in bullfrogs, *Lithobates catesbeianus*. We hypothesized that neurons of the locus coeruleus (LC) would respond intrinsically to hypercapnic acidosis (HA). Whole-cell patch-clamp electrophysiology was used to measure firing rates in excitatory (chemosensitivity index (CI)=185±62%), inhibitory (CI=40±25%) and non-chemosensitive (CI=110±9%) LC neurons in bullfrog brainstem slice preparations. 76% of neurons increased firing rates and while 7% and 17% were either inhibited or unstimulated, respectively, when exposed to HA. LC neurons increased firing rates during exposure to both small and large changes in dissolved CO_2 (ΔpH= 0.07-0.42U; $P<0.05$). During chemical synaptic blockade, half of chemosensitive LC neurons increased firing during HA. Uncoupling chemical synapses hyperpolarized the membrane of non-intrinsically chemosensitive neurons by 7±3mV while removing chemical synapses did not alter resting membrane potential ($P<0.05$). CI's for intrinsically chemosensitive and non-intrinsically chemosensitive LC neurons during synaptic block were 154±21% and 94 ±17% ($P<0.05$), respectively. Collectively, these data show that LC neurons intrinsically detect a wide-range of CO_2/pH changes and transduce these blood gas alterations into elevated firing responses in bullfrogs.

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Hypercapnia Inhibits a Transient K^+ Current in Chemosensitive Neurons from the Nucleus Tractus Solitarius (NTS) of Neonatal Rats

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Central chemosensitivity is believed to be mediated by CO_2/H^+-sensitive neurons within several brainstem regions. The increased firing rate of these neurons to elevated CO_2 (hypercapnia) involves membrane depolarization due to inhibition of K^+ channels by CO_2-induced acidification. We studied K^+ currents in neurons from brainstem slices of the NTS from neonatal rats (P3-P12) using whole cell voltage clamp techniques with aCSF containing tetrodotoxin (Na^+ channel blocker) and Cd^{2+} (Ca^{2+} channel blocker). Currents were induced by 10 mV voltage steps (300 ms) from a holding potential of -100 mV (range of -120 mV to +30 mV). Current traces showed a transient peak followed by a sustained current. A similar protocol, but with a -40 mV inactivating prepulse (200 ms) before the voltage steps, yielded a sustained current (delayed-rctifying K^+ (K_{DR}) current). The difference current was rapidly activating and inactivating (A current). In chemosensitive neurons (increased firing rate in response to hypercapnia, 15% CO_2), hypercapnia significantly inhibited (21%) the A current but had no effect on the K_{DR} current. In contrast, hypercapnia did not affect the A current of non-chemosensitive neurons (neurons whose firing rate did not change in response to hypercapnia). It has previously been shown that the chemosensitivity of NTS neurons can be blocked by 4-aminopyridine (4AP). We find that 4AP (5mM) inhibits the A current in NTS neurons by 57%. It also has a small inhibitory effect (37%) on the K_{DR} current. These data indicate that the A current plays a major role in the hypercapnia-induced enhanced activity of chemosensitive neuron in NTS. The difference in the effect of hypercapnia on the A current in different NTS neurons strongly suggests that different K^+ channel subunits make up the A current in chemosensitive vs. non-chemosensitive NTS neurons. *Supported by NIH Grant R01 HL-56683 & WSU Early Start/Augmentation Grant*.

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Hypercapnia Inhibits Both Transient and Sustained Potassium Currents in Chemosensitive Neurons from Neonatal Rat Locus Coeruleus (LC)

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Increased ventilation is stimulated by an increase in CO_2/H^+, which is detected by peripheral chemoreceptors and central chemosensitive neurons. We studied CO_2/H^+-sensitive K^+ channels in rat LC neurons. We hypothesize that multiple ion channels are involved in the neuronal response to changes in CO_2/H^+, with K^+ channels being an important component mediating this response. In this study, we used whole cell voltage clamp to test the effect of hypercapnia on a transient (I_A) and a TEA- sensitive (I_{KDR}) K^+ current and a TEA-insensitive sustained current of LC neurons from neonatal rat brain slices. Our results show that K_A and K_{dr} are activated at resting membrane potential. Five minutes after perfusion with aCSF equilibrated with 15% CO_2 (pH_o = 7.45), all three outwardly rectifying K^+ currents were significantly inhibited. The inhibition of I_A by HA was significantly reduced in isohydric hypercapnia (IH), where the extracellular pH was held constant at 7.45. These results suggest that hypercapnia increases the firing frequency of LC neurons through depolarization due to inhibition of K_A and K_{dr}, and decreased extracellular pH plays a vital role in the inhibition of K_A by hypercapnia.
Supported by NIH Grant R01-HL-56683-11.

Hands-on PhUn week activities at Medway Elementary School

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Medway Elementary School is in a rural district north of Dayton, OH where many students do not consider attending university in their future let alone pursuing a career in STEM. My students and I introduced the field of physiology to students in Medway using a combination of technical (models of lungs, hearts, *etc*., sphygmomanometers, skinfold calipers) and household items. After introducing the field of physiology and passing models around the classrooms, we had a student volunteer to be dressed as a scientist, and the whole class participated in determining which of a variety of items a scientist would wear or use. We discussed variability in tools use and stereotypes about scientists. We did several experiments with the students including the effects of exercise on heart rate and ventilation, tube diameter on rates of exhalation, shape on extensibility, surfactant on surface tension forces, chronic vs. intermittent muscle contraction on time to fatigue, and displacement of water to calculate lung volumes. We focused on the importance of quantification in science with the goal of encouraging study and application of mathematics by the students as they continue through middle and high school.

The American Physiological Society encourages all of you to participate in PhUn week:

http://www.the-aps.org/mm/Education/K-12/EducationProjects/PhUn-Week

Alterations in the ion channels SK3, HCN-1, and Kv2.1 in rodent lumbar α-motoneurons following peripheral axotomy

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Peripheral axotomy causes well-characterized alterations in intrinsic motoneuron (MN) activity and excitability including a decrease in rheobase and alterations in spike frequency and afterhyperpolarization (AHP) (Gustafsson, 1979; Kuno et al., 1974a,b). Alterations in expression, regulation and distribution of multiple ion channels likely underlie the physiological changes observed after axotomy. For example, dynamic modulation of Kv2.1 delayed rectifyer currents dramatically affects the intrinsic excitability of both mammalian and non-mammalian neurons (Mohapatra et al., 2009). Several studies suggest type-specific changes in AHP properties following peripheral axotomy, including a shortening of AHP duration in cells innervating slow twitch muscle fibers and a lengthening of AHP duration in cells innervating fast twitch muscle fibers (Kuno et al., 1974a,b; Gustafsson and Pinter, 1984). In motoneurons, small conductance Ca^{2+}-activated potassium (SK) currents and HCN mediated I_h (sag) currents contribute to firing rate by regulating and shaping the AHP and synaptic currents. Here, we used immunohistochemistry and *in vivo* electrophysiology to investigate the specific distribution and expression patterns of SK3, HCN-1, and Kv2.1 channel isoforms in physiologically identified rodent spinal MNs pre- and post-axotomy. In all MNs membrane expression of Kv2.1 is initially disrupted but returns to a pre-axotomy pattern independent of proper reinnervation of the peripheral target. In axotomized fast type motoneurons there is a prolonged increase in expression of SK3-IR and a decrease of HCN-1 expression, which is consistent with observed changes in AHP duration in this class of MN. These results implicate several specific ion channels as potentially important contributors to the complex physiological changes in axotomized motoneurons that may influence motoneuron intrinsic excitability and survival.

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Myocardial Angiotensin II Metabolism Analysis Using MALDI Imaging

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Mass spectrometric (MS) methods were used to study angiotensin (Ang) metabolism in mouse heart. The focus was on Ang II, Ang-(1-7) and Ang III. Goals were: 1) develop and optimize new technique for *in situ* assessment of Ang II processing within the myocardium using matrix-assisted laser desorption ionization (MALDI) imaging and 2) to validate results by *in vitro* MALDI MS enzyme assay as well as specific enzyme inhibitors. This newly developed imaging method has the advantage of allowing for regional spatial visualization and quantification of the formed peptides within the myocardium. Frozen mouse heart sections (12 µm) were incubated with 5 µL of 10-1000 ng/µL Ang II for 5-15 min at 37 ºC. The main formed peptides, Ang III and Ang-(1-7), were identified by MALDI-TOF/TOF. Ang-(1-7) was concentrated in the ventricle while Ang III was detected throughout the heart. For the MALDI MS enzyme assay, left ventricular (LV) homogenate was incubated with Ang II at 37 ºC. The reaction mixture was spotted on target plate and peptides were detected using MS. Results showed that Ang peptide formation was greatest at 100 ng/µL Ang II with short incubation period. Ang III formation was predominant with Ang III/ Ang II peak intensity 63%, whereas Ang (1-7)/ Ang II peak intensity was 22%. Ang III generation was blocked by the APA inhibitor, glutamate phosphonate (Glu-P), in a concentration dependent manner. The ACE2 inhibitor, MLN-4760, reduced myocardial Ang (1-7) formation by 66%. Interestingly, blockade of APA by Glu-P caused a 4 fold increase in Ang (1-7)/Ang II ratio. Results validated MALDI imaging for the *in situ* assessment of Ang II metabolizing enzymes. Ang II is mainly metabolized by APA in the myocardium while ACE2 is the main enzyme responsible for Ang-(1-7) formation from Ang II in the LV. This is the first report on localization of Ang II processing enzymes in the heart using MALDI imaging. This procedure can be exploited for the development of new therapeutic avenues that can be used to target the devastating Ang II effects within the myocardium.

TRPV4 channels mediate cardiac fibroblast differential by integrating mechanical and soluble signals

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The phenotypic switch underlying the differentiation of cardiac fibroblasts into hypersecretory myofibroblasts is critical for cardiac remodeling following myocardial infarction. Myofibroblasts facilitate wound repair in the myocardium by secreting and organizing extracellular matrix (ECM) during the wound healing process. However, the molecular mechanisms involved in myofibroblast differentiation are not well known. TGF-β has been shown to promote differentiation and this, combined with the robust mechanical environment in the heart, lead us to hypothesize that the mechanotransduction and TGF-β signaling pathways play active roles in the differentiation of cardiac fibroblasts to myofibroblasts. Here, we show that the mechanosensitive ion channel TRPV4 significantly inhibited TGF-β1-induced differentiation as measured by incorporation of α-SMA into stress fibers. Further, we found that TGF-β1-induced myofibroblast differentiation was dependent on ECM stiffness, a response that was attenuated by TRPV4 blockade. Finally, TGF-β1 treated fibroblasts exhibited enhanced TRPV4 expression and TRPV4-mediated calcium influx compared to untreated controls. Taken together these results suggest for the first time that the mechanosensitive ion channel, TRPV4, regulates cardiac fibroblast differentiation to myofibroblasts by integrating signals from TGF-β1 and mechanical factors.

Chromium downregulates a potent proatherogenic protein, thrombospondin-1, in vascular smooth muscle cells via reduced O- glycosylation and oxidative stress.

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Trivalent chromium (Cr^{3+}), a beneficial mineral nutrient required for regulation of insulin action, metabolic syndrome and cardiovascular disease is a proposed to have a therapeutic role in glycemic and cardiovascular health. Diabetic individuals typically have relatively low circulating level of Cr^{3+}, and Cr^{3+} deficiency is implicated as contributing factor in pathogensis of atherosclerosis and Coronary Artery Disease (CAD). Despite emerging evidence supporting a protective role for Cr^{3+} in atherosclerosis, the therapeutic potential of Cr^{3+} in diabetic vascular complications and the underlying mechanism(s) of Cr^{3+} action in vascular cells remain poorly studied. The present study is the first evidence that Cr^{3+} downregulates a potent proatherogenic and antiangiogenic matricellular protein thrombospondin-1 (TSP-1), previously linked to development of atherosclerotic lesions, CAD and Myocardial Infarction. Specifically, Cr^{3+} significantly decreased TSP-1 expression induced by high glucose or glycosylation activators in human aortic smooth muscle cells (HASMC). We reported earlier that glycosylation mediates TSP-1 upregulation by glucose. We now show that Cr^{3+}-mediated inhibition of TSP-1 is accompanied by decreased intracellular protein O-glycosylation, shown by immunoblotting and immunocytochemistry, coupled with a reduction in O-glcNAc transferase (OGT) expression, the enzyme catalyzing protein O-gylcosylation. TSP-1 expression was also previously reported to be upregulated by oxidative stress. In the present work, we found that Cr^{3+} TSP-1 expression in HASMC induced by a standard oxidant, H_2O_2. Furthermore, using XF24 Extracellular Flux Analyzer, we demonstrate that Cr^{3+} prevents high glucose-induced decrease in maximal oxygen consumption rate, an index for mitochondrial metabolic activity, suggesting a role to combat oxidative stress. Additionally, using fluorescence microscopy, we show that Cr^{3+} attenuates high glucose-induced oxidative stress in HASMC via reduction in reactive oxygen species (ROS).

Collectively, our data suggest that decreased protein O-glycosylation and attenuation of oxidative stress by Cr^{3+} downregulates TSP-1 providing a novel antiatherogenic effect of Cr^{3+} in diabetic atherosclerotic complications.

Knockout of type VI collagen preserves mitochondrial structure and function following myocardial infarction

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Cardiac remodeling is a dynamic process that is accelerated following myocardial infarction (MI) injury. The remodeling process, mediated chiefly by the cardiac fibroblasts and myofibroblasts, largely involves the deposition of extracellular matrix (ECM) at the injury site to form a scar and stabilize the ventricular wall. Previously, fibrillar collagens type I and III have been the focus of the remodeling field however, our lab has recently demonstrated a potentially novel role for non-fibrillar type of VI collagen (Col6) in remodeling. It is know that deficiencies in Col6 lead to age related skeletal muscle disorders such as Bethlem's myopathy that are characterized by skeletal muscle weakness and limb contracture which has been attributed to altered mitochondrial function in skeletal muscle, however the effects in the heart are not well known. Our lab was the first to report the effect of Col6 deficiency in cardiac muscle leading to a "paradoxical" improvement in post-MI remodeling and systolic function in $Col6^{-/-}$ mice. Now we direct our attention to investigating mechanisms by which the absence of Col6 confers protection against ischemic injury. *We hypothesize that the absence of Col6 improves mitochondrial morphology and preserves mitochondrial function post-MI*. To test this, we performed electron microscopy and measured mitochondrial respiration to identify structural and functional differences of mitochondria between WT and $Col6^{-/-}$ mice pre-and post-MI. Electron microgrpahs revealed enhanced mitochondrial fusion and increased glycogen deposition within myocytes of $Col6^{-/-}$ mice 3 days post-MI. $Col6^{-/-}$ mice demonstrated a lower respiratory control index (RCI) compared to WT mice (4.09±0.19 vs. 7.46±1.56) however, following MI (24 hrs.) the RCI of $Col6^{-/-}$ mice did not significantly change compared to $Col6^{-/-}$ shams while WT mice underwent a significant decline in RCI compared to shams. Together, these data indicate that the absence of Col6 preserves mitochondrial morphology and mitochondrial respiration following MI leading to improved remodeling and function.

Regulation of tumor angiogenesis by mechanosensitive TRPV4 channels

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Tumor vessels are characterized by abnormal morphology and structure that cause inefficient delivery of anti-cancer agents. We have previously shown that aberrant Rho-mediated mechanosensitivity of tumor-derived capillary endothelial (tumor CE) cells to mechanical cues is the underlying mechanism of tumor vessel malformations. Here, we show that expression and activity of a mechanosensitive ion channel, TRPV4, is significantly lower in tumor CE cells and that overexpression of TRPV4 restored their mechanosensitivity toward substrate elasticity. Further, TRPV4 overexpression inhibited abnormal migration and high Rho activity exhibited by tumor CE. Importantly, overexpression of TRPV4 alone normalized the aberrant capillary formation by tumor CE in 2D- and 3D-Matrigels. Finally, tumor xenograft experiments revealed that, compared to tumors in wild type mice, those in TRPV4 KO mice exhibit a greater degree of vascular abnormality, as demonstrated by increased vessel diameter and reduced pericyte coverage, as well as enhanced growth. This work, therefore, demonstrates the critical role for TRPV4 channels in tumor endothelial cell mechanosensitivity and offers a novel therapeutic target for normalizing tumor vessel malformations and improving anti-cancer drug delivery.

Alteration of Renal Neprilysin, Angiotensin Converting Enzyme (ACE2) and Nephrin in Streptozotocin (STZ) Diabetic Mice

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Diabetic nephropathy is one of the major microvascular complications of diabetes which eventually manifests into end stage renal disease. Alteration in renin-angiotensin system (RAS) is considered to be the primary cause underlying this implication. Emerging evidence suggests that the biological actions of Ang II may be opposed by the formation of Ang (1-7), partly generated by the actions of ACE2 and neprilysin (NEP). NEP is a zinc-containing metallopeptidase catalyzing the conversion of Ang I to Ang-(1-7), a potent vasodialator, thus counteracting the deleterious effects of Ang II. In our previous studies it has been shown that renal NEP is down-regulated and ACE2 is up-regulated in type 2 diabetic (db/db) mice. The goal of the present study is to explore the role of renal NEP and ACE2 in the pathogenesis of diabetic nephropathy in the STZ mouse model of type 1 diabetes. Diabetes was induced by five sonsecutive injections of STZ (50 mg/kp, i.p.). Urine was collected in presence of protease inhibitor for measuring albumin, creatinine and total protein contents. STZ diabetic mice exhibited hyperglycemia, microalbuminuria and renal hypertrophy. Renal NEP activity, detected via formation of Ang-(1-7) (m/z 899), was significantly reduced by 30% in diabetic mice. Renal ACE2 activity, measured using the fluorogenic test assay was found to be unaltered in diabetic and control mice. Western blot analysis demonstrated decreased renal NEP, nephrin and unaltered ACE2 protein expression in diabetic mice, which was further supported by immunohistochemical staining. In conclusion, decreased NEP coupled with depletion of nephrin in diabetes could possibly play a crucial role in the development of diabetic nephropathy.

Ohio Physiological Society 2012 Meeting Attendees

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