“FROM FORM TO FUNCTION”

23rd Annual Meeting

Ohio Physiological Society

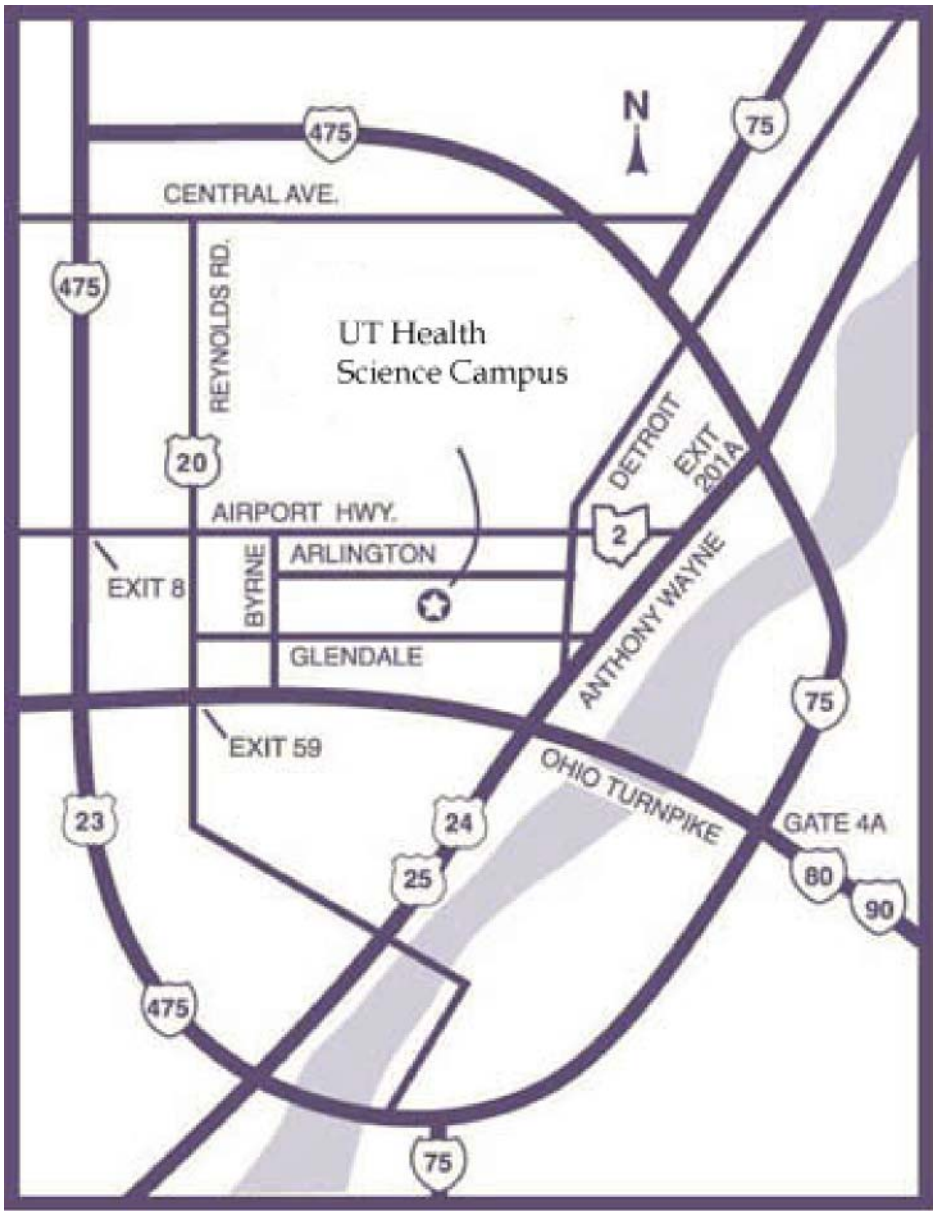
A chapter of the American Physiological Society

&

University of Toledo
Health Science Campus

Eleanor N. Dana
Conference Center
University of Toledo
Health Science Campus
Toledo, Ohio

November 6-7, 2008



From I-75/475 Northbound or US 23/475 Southbound:
Use Exit 8 (Airport Highway, State Route 2). Proceed east on Airport Highway three miles to Byrne Road. Turn Right on Byrne Road and proceed to Arlington Avenue (first traffic signal). Turn left on Arlington and proceed to UT Health Science Campus entrance. Turn right on Hospital Drive.

From I-75 Southbound:
Use exit 201A (Maumee/U.S. 25) to Anthony Wayne Trail. Go south on Anthony Wayne Trail to Glendale Avenue. Turn right on Glendale Avenue and proceed west to MUO Boulevard, (third light) and turn right.

From Ohio Turnpike, East or Westbound:
Use Exit 59 for Maumee and Toledo; head toward Toledo from toll booth and proceed north one mile on Reynolds Road (U.S. 20) to Glendale Avenue. Turn right on Glendale Avenue. Drive three miles to MUO Boulevard (fifth light) and turn left.

University of Toledo
Health Science Campus
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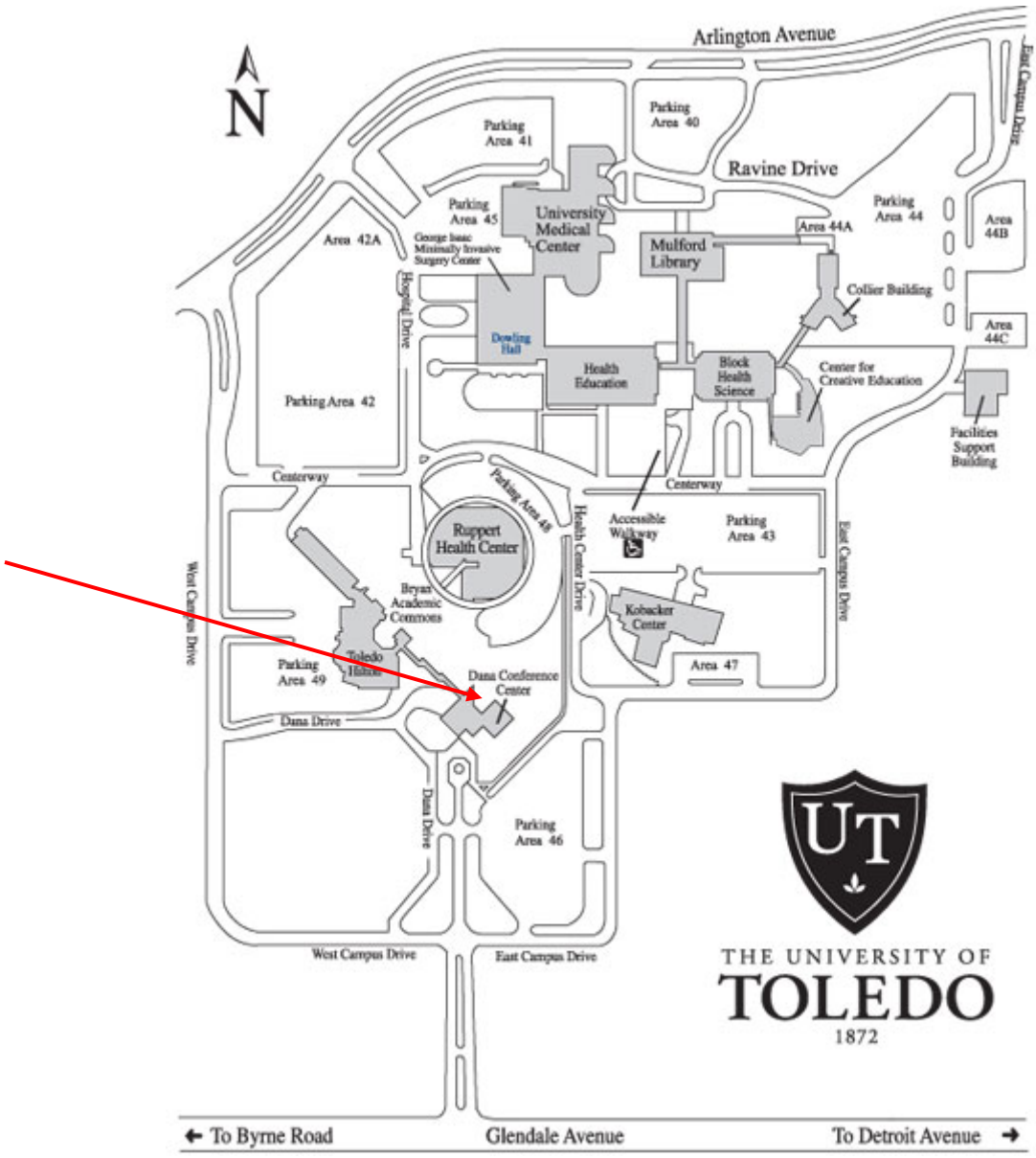

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23rd Annual Meeting, November 6-7, 2008

Venue: Dana Conference Center

University of Toledo Health Science Campus, Toledo, Ohio

Agenda

Thursday, November 6, 2008

10:00 AM – 4:00 PM	Golf Outing (Organizer: **Maurice Manning, Ph.D., D.Sc.**, Professor of Biochemistry and Cancer Biology)
2:00 PM	Poster set-up (Allen/Erie Rooms-See top right corner of abstract)
4:30 PM	Registration
5:00 - 6:00 PM	Session I: Panel discussion with physiologists (**APS PhUn Week** activity/ High School Students only)
5:00 - 6:15 PM	Open display of posters (Allen/Erie Rooms)
6:15 PM	Welcome **Michael Bisesi, Ph.D.**, Senior Associate Dean College of Graduate Studies, University of Toledo
6:30 PM	**Joseph Nadeau, Ph.D.** Professor and Chairman, Genetics Case Western Reserve University **"Genetic and Systems Studies of Metabolic Diseases"**
7:30 - 9:30 PM	Cash Bar
8:00 PM	Dinner (Birch/Elm Rooms, Hilton)

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Friday, November 7, 2008

8:00 AM	Continental Breakfast (Dana Center Atrium)
8:30 AM	Registration
8:50 AM	Opening Remarks (Presentation of iPOD winner)
9:00 -10:00 AM	Gale Davy, Director, Outreach & Advocacy Americans for Medical Progress, Alexandria, VA **"Public Policy - The Critical Role of Scientists"**
10:00 - 11:30 AM	**Poster Presentations** (Allen/Erie Rooms-see top right corner of abstract)
	Session II: Oral Presentations
11:30 - 11:45 AM	Ying Xu - Ohio State University "Eccentric Contraction In *mdx* Mice Trabeculae"
11:45 - 12:00	Ameet Chimote - Wright State University "A Comparison of the Cellular Response to Hyposmotic and Apoptotic Stress in A Human Lens Epithelial Cell Line (HLEC-B3)"
12:00 - 12:15 PM	Edward Toland - University of Toledo Health Science Campus "Multiple Quantitative Trait Loci on Rat Chromosome 9 In Which Spontaneously Hypertensive Rat Alleles Confer A Reduction In Blood Pressure Of A Salt-Sensitive Model"
12:15 - 12:30 PM	Patrick Mineo - Miami University "Skeletal Muscle Energetics Following Cold Acclimation in A Brown Adipose Tissue Deficient Mouse"
12:30 - 2:00 PM	Lunch (Birch/Elm Rooms, Hilton)
	Session III: Oral Presentations
2:00 - 2:15 PM	Patricia Shamhart - NEOUCOM "Angiotensin II-Induced ERK1/2 Activation Is Mediated By Pkcδ and Intracellular Calcium in Cardiac Fibroblasts"
2:15 - 2:30 PM	Kaylan Haizlip - Ohio State University "Functional and Molecular Changes During Development Of Compensatory Hypertrophy"
2:30 - 2:45 PM	Gargi Roy - University of Toledo Health Science Campus "The Mechanism of Defective ER To-Golgi Trafficking Of ΔF508 CFTR"
2:45 - 3:00 PM	Coffee Break
3:00 - 4:00 PM	Muthu Periasamy, Ph.D. Professor and Chairman, Physiology and Cell Biology Ohio State University **"Cardiac SR Ca^{2+} ATPase and Its Role in Heart Disease"**
4:00 PM	Presentation of the Peter Lauf Travel Scholarship Award
4:15 PM	Concluding Remarks - Dr. Peter Lauf, Founding President OPS
4:30 PM - 5:30 PM	Business Meeting of the OPS Executive Committee

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MECHANICAL PROPERTIES OF ATRIAL MUSCLE PREPARATIONS

Jessica L. Abraham, Paul M.L. Janssen
Department of Physiology and Cell Biology, The Ohio State University
Correspondence to: janssen.10@osu.edu

The force frequency relationship (FFR) is an important intrinsic regulator of cardiac muscle function. The FFR is positive, more force at higher frequencies, in mammals within their respective normal range of heart rates. Therefore, in normal myocardium the FFR is positive while in heart failure this relationship is altered and the FFR becomes blunted or even negative. While the ventricles remain important in heart failure, little is known about the function and mechanics of the atria both in normal and in the disease model. In our current study, we are attempting to examine the mechanics of both the right and left atria. Ultra-thin right and left atrial trabeculae were isolated from New Zealand White rabbit hearts and attached between a force transducer and a micromanipulator. The isolated muscle was stretched to an optimal preload, the FFR was established on each muscle by stimulating the muscle to twitch at 1, 2, 3, and 4 Hz respectively, and an isoproterenol-response curve was established using 1nM to 1μM. The FFR of the right atria reaches a maximal force at 2 Hz. However, the FFR of the left atria does not reach maximal force until 3 Hz. The relaxation time and time to peak kinetics were similar between the right and right atria as well as the response to isoproterenol. This data suggests that there are both similarities and differences in the mechanical properties of the right and left atria. The findings of this study are beneficial for furthering our knowledge about how the heart functions normally so that we can, in turn, learn more about the heart in heart failure.

Li FLUXES REVEAL PRESENCE OF Na-Cl COTRANSPORT (NCC) IN HUMAN LENS EPITHELIAL CELLS (LECS)

Norma C. Adragna[1,3], Ameet A. Chimote[1], and Peter K. Lauf [1,2]
[1]Cell Biophysics Group, [2]Department of Pathology, [3]Department of Pharmacology and Toxicology, Wright State University Boonshoft School of Medicine, 3640 Colonel Glenn Hwy., Dayton, OH, 45435, USA.

Na-K-2Cl cotransport (NKCC) moves RbCl/KCl+NaCl/LiCl and, in FHL124 LECs, is not inhibited by hypotonicity. Here, we studied Li fluxes through NKCC in hyposmotic high K media and Rb fluxes in hyposmotic high Na to understand this effect. Li and Rb uptake were measured by atomic absorption spectrophotometry to determine LiKCC or NRbCC through bumetanide-sensitive (BS) and Cl-dependent fluxes in Na/K or Na-free/K-free (N-methyl-D-glucamine) and Cl or Cl-free (sulfamate or nitrate) media ± ouabain (O) or [O+B], ± thiazides, at varying Rb, Li or Cl molar fractions (MF). Main findings are: Li influx in isosmotic (300 mOsM) K was 1/3 of Rb influx in Na, and was mainly mediated by a Cl-dependent Li flux (LiKCC). In 200 mOsM high K, LiKCC was abolished, whereas in high Na, NRbCC remained active. LiKCC and BS-Li influx (BS-LiKCC) showed bell-shape curves for 0.1-1 Li MF, maxing at ~0.6 MF. BS-LiKCC was ¼ of LiKCC. The difference, i.e. the B-insensitive/Cl-dependent-Li influx, saturated with Li and Cl MFs. K_{ms} for Li were 11 with and 7-8 mM without external K, respectively; and ~40 mM for Cl. Neither furosemide (<100 μM) nor thiazide derivatives inhibited LiCC. RTPCR, Western blots and immunochemistry revealed NCC RNA and protein, with NCC-specific antibodies, besides the expected NKCC1. Thus, a K-independent/Cl-dependent Li flux (LiCC) revealed the presence of NCC in FHL124 LECs.

Hsp90-DEPENDENT AND INDEPENDENT FUNCTIONS OF FKBP8 IN THE MATURATION OF CFTR

Yeshavanth K. Banasavadi-Siddegowda*, Junbo Mai, Elaine Chalfin, Xiaodong Wang.
Department of Physiology & Pharmacology. University of Toledo College of Medicine, Cardiovascular and Metabolic Diseases Track.

Ion channels play important roles in cardiovascular physiology. Defective exocytic trafficking can cause loss of function at the cell surface. These are the basis of a number of cardiovascular diseases such as long QT syndrome. We use cystic fibrosis transmembrane conductance regulator (CFTR), a chloride channel present in many tissues including cardiac tissues as a model cargo protein to understand how cellular chaperone machinery mediates the maturation and export of ion channels.

The loss of cell surface chloride conductance is the hallmark of cystic fibrosis (CF). The most common CF mutation is the deletion of phenylalanine at position 508 of CFTR (ΔF508). Instead of producing a non-functional chloride channel, ΔF508 specifically prevents the export of CFTR from the endoplasmic reticulum (ER) leading to degradation. FK506-binding protein 8 (FKBP8), a member of the immunophilin family, was identified together with multiple chaperones and co-chaperones to associate with the ER-localized CFTR. FKBP8 contains an N-terminal region of unknown function, an FK506-binding peptidylprolyl cis/trans isomerase (PPIase) domain, a tetratricopeptide repeat (TPR) domain, a calmodulin binding motif (CaM), and a Carboxy terminal transmembrane (TM) domain. Overexpression of FKBP8 in HEK293 cells stably expressing ΔF508 destabilizes CFTR in the ER.

We observed differential effects of FKBP8 overexpression on the export and degradation of CFTR in a dose-dependent manner. FKBP8 associate with Hsp90 through its TPR domain. To probe the role of FKBP8 as Hsp90 co-chaperone in CFTR maturation, we constructed mutant FKBP8 that fails to associate with Hsp90. At low level of overexpression (~10 fold) FKBP8 stabilizes the ER form of CFTR in an Hsp90-independent manner. At medium level of overexpression (~29 fold), FKBP8 specifically inhibit export of wildtype CFTR which is independent of Hsp90 association whereas, it has different effect on stabilization of ER form of CFTR in wildtype and ΔF508 which is dependent on Hsp90 association with TPR domain. At high level of overexpression (~33 fold) FKBP8 destabilizes CFTR in the ER; Disruption of Hsp90 association further promotes such destabilization in wildtype CFTR.

Our results indicate that FKBP8 regulate the folding of wildtype and ΔF508 CFTR differently. It has both Hsp90-dependent & -independent functions. Further studies aimed at revealing the role of other domains of FKBP8 in CFTR export and stability would present a complete picture of its role in CFTR maturation.

Supported by American Heart Association.

REGULATION OF INTRACELLULAR CHOLESTEROL DISTRIBUTION BY THE Na/K-ATPase

Yiliang Chen,[1] Haojie Wang,[1] Ting Cai,[1] Zhichuan Li,[1] Elizabeth Loreaux,[2] Jerry B Lingrel,[2] Zijian Xie[1]
[1]Department of Physiology and Pharmacology, College of Medicine, University of Toledo, Toledo, Ohio 43614-2598; [2]Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati, College of Medicine, Cincinnati, Ohio 45267
Corresponding Author, email: Yiliang.Chen@utoledo.edu

The Na/K-ATPase was discovered as an energy transducing ion pump. Recent studies have ascribed many non-pumping functions to the Na/K-ATPase. We showed here that graded knockdown of the cellular Na/K-ATPase produced a parallel decrease in cholesterol in the caveolar fractions of LLC-PK1 cell lysates. This observation was further substantiated by imaging analysis, showing redistribution of cholesterol from the plasma membrane to intracellular compartments in the knockdown cells. Moreover, this regulation was confirmed in the Na/K-ATPase α1+/- mouse liver. Functionally, the Na/K-ATPase knockdown decreased cholesterol content in the endoplasmic reticulum (ER), resulting in an activation of the sterol regulatory element binding protein 2 (SREBP2) and a subsequent increase in the expression of HMG-CoA reductase and the low density lipoprotein (LDL) receptor in the liver. Consistently, the knockdown caused a modest increase in hepatic cholesterol as well as a reduction in plasma cholesterol. Furthermore, we presented evidences that Na/K-ATPase α1 subunit directly interacted with cholesterol revealed by fluorescence energy transfer (FRET) analysis, which may represent an important sensing mechanism by which the cells regulate ER cholesterol and then the SREBP2 pathway.

A COMPARISON OF THE CELLULAR RESPONSE TO HYPOSMOTIC AND APOPTOTIC STRESS IN A HUMAN LENS EPITHELIAL CELL LINE (HLEC-B3)

Ameet A. Chimote[1], Norma C. Adragna[1, 2] and Peter K. Lauf[1]
[1] Cell Biophysics Group, Departments of [2] Pharmacology and Toxicology, Wright State University, Boonshoft School of Medicine, Dayton OH 45435. For correspondence, contact Dr. Peter K. Lauf at peter.lauf@wright.edu

Cell volume regulation to maintain volume constancy (VC) is an important physiological process in all living cells. VC is achieved during physiological stress by altering trans-membrane ion and water flow. Cell volume decrease responses due to hyposmotic stress and a pro-apoptotic signal were studied and characterized in cultured HLEC-B3. Intracellular K content (K_i) was measured by atomic absorption spectrophotometry, intracellular Cl concentration $[Cl]_i$ with the chloride sensitive fluorescent dye MQAE by a double ionophore technique, while intracellular water was determined gravimetrically. Apoptosis was demonstrated using Cell Death Detection ELISA (CDDE) kit, which quantifies DNA fragmentation.

For hyposmotic stress, HLEC-B3 were exposed to 300 mOsM (isosmotic) and 150 mOsM (hyposmotic) balanced salt solution (BSS) as described previously (Lauf, P., Misri, S., Chimote, A., & Adragna, N. (2008). *Apparent intermediate conductance K channel hyposmotic activation in human lens epithelial cells. American Journal of Physiology. Cell Physiology*, *294*(3), C820-32). As compared to isosmotic media, cell water increased in hyposmotic media about 2-fold in the first 5 min, consistent with hyposmotic swelling of HLEC-B3, after which cell water fell within 30 min to below 20% of the baseline values commensurate with regulatory volume decrease (RVD). The efflux rate constant for cell water was ~0.04/min. Simultaneously K_i fell in the first 30 min by 42% with a rate constant of 0.04/min, as compared to the efflux rate of 0.004/min for isosmotic media. $[Cl]_i$ fell to 58% of baseline values after 15 min, reflecting dilution of the dye and not the actual Cl^- loss.

Significant apoptosis was induced in HLEC-B3 after treatment with 2μM staurosporine (STP) for 2 h in 300 mOsM media, as quantified by CDDE. No significant water loss was detected for the first 15 min after STP treatment, and 20% water loss at 30 min after which cell water returned to baseline values between 30 and 45 min. The efflux rate of water for the first 45 min was almost negligible and that of K efflux <0.007/min. $[Cl]_i$ fell to 25% of baseline values in the first 30 min and 19% between 30-60 min with an overall rate of ~0.004/h suggesting transient $[Cl]_i$ changes.

Results demonstrate that K, water and most likely Cl loss differ by an order of magnitude in hyposmotically induced RVD as compared with the early responses of K and water loss following apoptotic stress, suggesting that apoptotic volume decrease (AVD) occurs after 30 min whereas RVD is elicited within 10 min.

EPISTATIC GENETIC DETERMINANTS OF BLOOD PRESSURE AND MORTALITY IN A SALT-SENSITIVE HYPERTENSION MODEL: EFFECTS ON CARDIAC AND RENAL FUNCTION.

[1]George T. Cicila, [1]Eric E. Morgan, [1]Soon Jin Lee, [1]Edward J. Toland, [1]Phyllis Farms, [1]Shane Yerga-Woolwine, [2]Keith Bohman, [3]Andrea L. Nestor, [4]Sadik A. Khuder, and [1]Bina Joe
Departments of [1]Physiology and Pharmacology, [2]Pathology, [3]Surgery, and [4]Medicine
University of Toledo College of Medicine, 3035 Arlington Avenue, Toledo, Ohio 43614
george.cicila@utoledo.edu

While genetic determinants that protect against developing morbid conditions such as elevated blood pressure (BP) have been well investigated, much less is known regarding their impact on mortality. We concomitantly assessed rat chromosome 3 and 7 (RNO3 and RNO7) regions carrying genetic determinants of BP without known epistasis, for 1) their independent and combinatorial effects on BP and 2) the presence of genetic determinants of survival using Dahl Salt-sensitive (S) rat strains carrying Dahl Salt-resistant (R)-rat RNO3 and RNO7 congenic segments. While congenic and bicongenic S.R strains carried independent BP QTLs within the RNO3 and RNO7 congenic regions, only the RNO3 congenic segment contained allele(s) independently affecting mortality. The S.R bicongenic strain (RNO3+RNO7) demonstrated epistasis between R-rat RNO3 and RNO7 alleles for BP and survival under salt loading, with less than additive effects for BP, but more than additive for mortality. Additional experiments indicated that increased survival of RNO3+RNO7 bicongenic, compared to RNO3 congenic, rats was attributable, at least in part, to the ability of bicongenic rats to maintain lower BP despite prolonged exposure to an increased dietary salt (4% NaCl) intake. While no significant differences in echocardiographic measures were observed between RNO3 congenic and RNO3+RNO7 bicongenic rats, R-rat RNO3 congenic interval alleles were significantly associated with multiple measures of superior systolic function. Several echocardiographic measures, including relative wall thickness, showed epistasis between the products of RNO3 and RNO7 congenic interval alleles. A trend toward increased interstitial fibrosis was also observed in left ventricles of congenic and bicongenic rats, compared to S rats. The effects of RNO3 and RNO7 congenic interval alleles on renal function were dependent upon dietary NaCl intake, with superior renal function associated with R-rat RNO7 alleles on a baseline, low salt (0.3% NaCl) diet and with R-rat RNO3 alleles after chronic (4 week) exposure to a higher salt (2% NaCl) diet. These results are consistent with our hypothesis that interactions between alleles in different BP QTL-containing regions influence both BP and survival under salt-loading conditions and are traceable using S.R congenic strains as genetic tools. This is the first report of simultaneous detection of both independent and epistatic loci dictating, in part, mortality in a hypertensive rat strain.

ALPHA-TOCOPHEROL PROTECTS HUMAN AIRWAY EPITHELIAL CELLS AGAINST CADMIUM- AND CIGARETTE SMOKE-INDUCED INHIBITION OF THE CFTR CHLORIDE CHANNEL

Jessica Rennolds, Susie Butler, Kevin Maloney, Daren Knoell, Narasimham Parinandi, and Estelle Cormet-Boyaka.
Department of Internal Medicine, Division of Pulmonary, Critical Care and Sleep Medicine, The Ohio State University, Columbus, OH 43210, USA.

The Cystic Fibrosis Transmembrane conductance Regulator (CFTR) is a chloride channel that is mostly expressed in epithelial cells. In the lung, CFTR plays an important role in the hydration of the layer of liquid that lines the airway surface. Mutations in the *cftr* gene, that affect CFTR trafficking and/or function, lead to Cystic Fibrosis that is characterized by chronic lung inflammation. Cadmium is a toxic heavy metal present in the environment and cigarette smoke. Its inhalation is associated with decreased pulmonary function, lung cancer and chronic obstructive pulmonary disease. Cigarette smoke has been recently reported to suppress the expression of the CFTR chloride channel. Therefore, we investigated the effect of cadmium on the CFTR chloride channel using human airway epithelial cells. We found that cadmium decreases the expression of the CFTR protein resulting in a reduction of chloride transport in the bronchial epithelial cells Calu-3. Since cadmium can induce the production of reactive oxygen species we tested the effect of antioxidants on CFTR protein. Vitamin E prevented the inhibitory effect of cadmium on CFTR expression and function in Calu-3 cells and primary human airway epithelial cells. We found that cadmium decreased the content of polyunsaturated fatty acids that was prevented by addition of vitamin E suggesting that lipid peroxidation affects CFTR protein. Our findings identify cadmium as a potent inhibitor of the CFTR chloride channel in lung epithelial cells. Since high levels of cadmium are present in mainstream and sidestream cigarette smoke, as well as contaminated air, it is important to better understand the effect of cadmium on epithelial cells.

CaMKII INHIBITION REVERSES BENZODIAZEPINE-INDUCED REDUCTION OF GABA SENSITIVITY AT SINGLE $GABA_AR$ CHANNELS

P. Das[1*], L.J. Greenfield Jr.[1,2], E.I. Tietz[1].
Dept. of Physiology and Pharmacology[1], Dept. of Neurology[2], Univ. of Toledo College of Medicine, Toledo, OH 43614 Corresponding: Paromita.Das@utoledo.edu

Two days after 1-week oral treatment with the benzodiazepine (BZ), flurazepam (FZP), L-type voltage-gated channel (L-VGCC) whole-cell Ca^{2+} current density is doubled and γ-amino butyric acid type A receptor (GABAR) single-channel open probability (Po) is reduced in cell-attached patches from dissociated hippocampal CA1 neurons. Blockade of Ca^{2+} influx by systemic injection of the L-VGCC antagonist, nimodipine 1 day after FZP treatment, restored sensitivity of FZP neurons to 5 μM GABA, increasing Po from 0.04 to 0.54 after 2 days to near control levels (0.68). We hypothesized that BZ-induced elevation of $[Ca^{2+}]i$ may suppress GABAR function by activating Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII). Single-channel recordings were obtained after slice preincubation with membrane-permeant CaMKII inhibitors. At 1 μM GABA, Po in control patches was 0.19 while no openings were observed in FZP patches. After pre-incubation with KN93, Po in FZP patches was 0.73. No openings were seen with the inactive analog KN92. Pre-incubation with mAIP, activated 'silent' single channels in FZP patches (Po=0.69) and increased the basal response of control patches (Po=0.57). Membrane-impermeant AIP and the PKC inhibitor, chelerythrine had no effect. Persistent activation of CaMKII is required to maintain basal GABAR function and for the BZ-induced, negative modulation of GABAR function associated with BZ tolerance.

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β2 INTEGRINS CONTRIBUTE TO SKELETAL MUSCLE HYPERTROPHY IN MICE

Christopher L. Dearth, Joseph S. Marino, Brian J. Tausch, Marc V. Manacci, Thomas J. McLoughlin, Samuel J. Rakyta, Matthew P. Linsenmayer, and Francis X. Pizza

Department of Kinesiology, The University of Toledo, 2801 West Bancroft Street, Toledo, OH, 43606 Corresponding author email: Francis.Pizza@utoledo.edu

We tested the contribution of β2 integrins, which are important for normal function of neutrophils and macrophages, to skeletal muscle hypertrophy after mechanical loading. Using the synergist ablation model of hypertrophy and mice deficient in the common β subunit of β2 integrins (CD18-/-), we found that overloaded muscles of wild type mice had greater myofiber size, dry muscle mass, and total protein content compared to CD18-/- mice. The hypertrophy in wild type mice was preceded by elevations in neutrophils, macrophages, satellite cell/myoblast proliferation (BrdU+desmin+ cells), markers of muscle differentiation (MyoD1 and myogenin gene expression and formation and size of regenerating myofibers), signaling for protein synthesis (phosphorylation of Akt and p70S6k), and reduced signaling for protein degradation (decreased gene expression of MAFbx/atrogin-1). The deficiency in β2 integrins however, altered the accumulation profile of neutrophils and macrophages, disrupted the temporal profile of satellite cell/myoblast proliferation, reduced markers of muscle differentiation, and impaired p70S6k signaling, all of which could serve as mechanisms for the impaired hypertrophy in overloaded CD18-/- mice. In conclusion, our findings indicate that β2 integrins contribute to the hypertrophic response to muscle overload by temporally regulating satellite cells/myoblast proliferation and by enhancing muscle differentiation and p70S6k signaling.

FoxO1 INHIBITS SKELETAL MUSCLE HYPERTROPHY

Alissa D. DeLong[1], Z. Brinkman[1], B. Renwand[1], Y. Kamei[2,3], S. Muira[2], O. Ezaki[2], and T. J. McLoughlin[1]
[1] The University of Toledo, [2] National Institute of Health and Nutrition, Shinjuku-ku, Tokyo, [3]Tokyo Medical and Dental University, Chiyoda-ku, Tokyo
Corresponding Author email- thomas.mcloughlin@utoledo.edu

The Akt/mTOR signaling pathway plays a key role in the regulation of skeletal muscle size. The FoxO1 transcription factor, a downstream target of Akt, has been shown to suppress key growth molecules (e.g. p^{70s6} kinase) within the Akt/mTOR pathway, indicating that FoxO1 may negatively regulate muscle hypertrophy through suppression of anabolism. To examine the role of FoxO1 in the regulation of skeletal muscle hypertrophy, wildtype (WT) and FoxO1overexpressing mice (FoxO1+/-) were exposed to synergist ablation (SA) or control (CT) surgery. After 14 days of CT or SA, plantaris muscles were assayed for changes in mass, protein content, cross-sectional area (CSA) and expression of total and phospho-Akt and -$p70^{s6k}$ via western blot analyses. Changes in MAFbx gene expression were assessed via qPCR. Although both strains exhibited significant increases in mass, protein content, and CSA after SA, increases were significantly greater in WT vs. FoxO1+/- (~60% vs. ~20%). SA resulted in increased total Akt in both strains; however, total Akt levels were higher in FoxO1+/- CT and SA vs. WT. Phospho-Akt (Ser473) was significantly greater in CT muscles of FoxO1+/- vs. WT mice. After SA, WT mice exhibited significant increases in phospho-Akt vs. CT, but FoxO1+/- mice exhibited no further gains in phospho-Akt. Total $p70^{s6k}$ was not different between CT muscles of WT and FoxO1+/- mice and although total $p70^{s6k}$ increased after SA in both strains there was no significant difference between strains. Phospho-$p70^{s6k}$ (Th389) was elevated in both strains after SA; however, WT mice exhibited greater phospho-$p70^{s6k}$ than FoxO1+/-. MAFbx gene expression was elevated in FoxO1+/- vs. WT mice and was suppressed in both strains after SA. These findings provide support that FoxO1 plays a strong role in the inhibition of skeletal muscle hypertrophy through the suppression of anabolic cell signaling.

DIRECT INHIBITION OF RECOMBINANT L-TYPE VOLTAGE-GATED CALCIUM CHANNEL CURRENTS BY BENZODIAZEPINES

Damien E. Earl*[1], John D. Franklin[1], Nathalie Boulineau[2], Elizabeth I. Tietz[1]
Departments of [1]Physiololgy and Pharmacology and [2]Neurology
University of Toledo College of Medicine, Toledo, OH, 43615
**damien.earl@utoledo.edu*

Benzodiazepines (BZs) are clinically used as hypnotics, anxiolytics, and anticonvulsants, but chronic BZ use increases the risk of physiological dependence manifested by a withdrawal syndrome, limiting their clinical usefulness. Using a 1-week oral flurazepam (FZP) treatment, which reliably produces anxiety-like behavior upon withdrawal, we detected an ~2-fold increase in Ca^{2+} current density through high voltage-gated calcium channels (VGCCs) in hippocampal CA1 neurons up to 2 days after withdrawal. Functional upregulation of L-type VGCCs may contribute to withdrawal anxiety, as prior injection of nimodipine (NIM), an L-VGCC antagonist, prevented anxiety *in vivo*. Due to structural similarities of BZs with dihydropyridine (DHP) L-VGCC antagonists, BZs may directly interact with L-VGCCs. Human embryonic kidney (HEK 293T) cells were transfected with α_1 ($Ca_v1.2$ or $Ca_v1.3$), β_3 and $\alpha_2\delta$-1 L-VGCC subunits and red fluorescent protein. The concentration-, voltage-, and use-dependent effects of BZs on whole-cell recordings of L-VGCC Ba^{2+} and Ca^{2+} currents were evaluated. BZs dose-dependently inhibited peak Ba^{2+} currents elicited at -25 mV in $Ca_v1.3$-containing channels [1-100 μM diazepam (DZP) up to 30±2%, n=4; 1-500 μM FZP up to 45±6%, n=2; compared to DHPs: 1 μM NIM, 71±3%, n=3; 1 μM nitrendipine, 44±4%, n=3]. BZs may enhance the inactive state of the channel, as steady-state currents were inhibited more than peak currents (100 μM DZP, 58±2%, $p<0.01$; 500 μM FZP, 56±4%, $p>0.05$ relative to peak inhibition reported above). In addition, DHP and BZ inhibition was generally greater at more positive membrane potentials at which more channel inactivation occurs. Recent studies have also shown that the degree of BZ inhibition increases with the frequency of channel activation. As with the DHPs, FZP has a greater capacity to inhibit the other L-VGCC neuronal α_1 subtype, $Ca_v1.2$ (1 μM NIM, 98±2%, n=2 vs. 92±4, n=3, $p>0.05$; 1 μM FZP, 21±2%, n=4 vs. 4±5%, n=2, $p<0.05$; 100 μM FZP, 79±2%, n=4 vs. 39±8%, n=2, $p<0.01$, measured at steady-state Ba^{2+} currents elicited at +5 mV in $Ca_v1.2$ vs. $Ca_v1.3$, respectively). These data suggest that a greater frequency and strength of depolarization could lead to more pronounced inhibition of L-VGCCs by BZs at the ~1 μM concentration found *in vivo* during chronic treatment, especially of $Ca_v1.2$-containing VGCCs.

Support: NIDA RO1-DA04075 and RO1-DA184342 (EIT), and UT predoctoral fellowships (DE).

CLONING AND EXPRESSION OF AQUAPORIN IN THE ANTENNAL GLAND OF CRAYFISH, *Procambarus clarkii*

Yang Gao, Yongping Gao and Michele G. Wheatly
Department of Biological Sciences, Wright State University 3640, Colonel Glenn Highway - Dayton, Ohio - 45435 Corresponding author: gao.7@wright.edu

Aquaporins (AQPs), membrane proteins, are responsible for transferring water as well as other small molecules such as glycerol and urea across the cell membrane. Up to now, AQP has been cloned from a variety of species including mammal, invertebrate, plants and bacteria. However, little is known in aquatic crustaceans. The crayfish, *Procambarus clarkii*, which has a discontinuous growth pattern (molting cycle), served as an ideal model to study the Ca transporting proteins in the past. The objective of this study is to examine the expression and the function of aquaporins in crayfish during molting. The crayfish antennal gland, a primary site of water loss and salt conservation, plays an important role in regulating the concentration of nutrients, solutes and ions. As a result, a partial AQP sequence has been cloned from the crayfish antennal gland. The expression level of the AQP in the antennal gland was compared to other epithelia tissues: gill and liver and non-epithelia tissue: tail muscle by using real-time PCR.

EFFECT OF COLD ACCLIMATION ON THE EXPRESSION OF SARCOPLASMIC CALCIUM BINDING PROTEIN (pcSCP1) VARIANTS IN THE FRESHWATER CRAYFISH, *Procambarus clarkii.*

Gillen, C.M., White, A.J., Northcutt, M.J., Gao, Y., Wheatly, M.G.
Kenyon College, Gambier, OH and Wright State University, Dayton, OH. Corresponding author: gillenc@kenyon.edu

We have investigated the effect of cold exposure on sarcoplasmic calcium binding protein (pcSCP1) from the freshwater crayfish, *Procambarus clarkii*. pcSCP1 is an EF-hand calcium binding protein that is highly expressed in invertebrate muscles where it acts as a calcium buffer. Sequencing of full length pcSCP1 clones revealed three variants of pcSCP1 (pcSCP1a, pcSCP1b, and pcSCP1c) that are identical except for a variable region between base pairs 222 and 345 of the 579 base pair open reading frame. We assessed expression of pcSCP1 by real-time PCR with variant specific primers using the relative quantification method and 18s ribosomal RNA as an internal calibrator. Expression of all three variants was highest in axial muscle, more than 10,000 fold above hepatopancreas. pcSCP1c was the primary variant in cardiac muscle where it was expressed about 10 fold above hepatopancreas. To evaluate expression during cold acclimation, crayfish were held at 4 degrees Celsius for 7 days and a control group was kept at room temperature. Heart SCP1 was found to be dramatically downregulated by cold exposure; all three variants were undetectable after cold exposure. In contrast, pcSCP1b and pcSCP1c were moderately upregulated in axial muscle after cold acclimation. (Funded by NSF 0445202 and Kenyon College).

FUNCTIONAL AND MOLECULAR CHANGES DURING DEVELOPMENT OF COMPENSATORY HYPERTROPHY

Kaylan M Haizlip, Tepmanas Bupha-Intr, Paul M L Janssen
The Dorothy M. Davis Heart and Lung Institute
Intergrated Biomedical Science Program, Department of Cell Biology and Physiology, and The Ohio State University, Columbus, Ohio 43210 Corresponding author: haizlip.4@osu.edu

Heart failure is often preceded by hypertrophy, in which the heart wall thickens via growth of the individual myocytes. It is currently understood that the progression to heart failure involves two steps: compensatory hypertrophy, a process where contractile function is enhanced and myocyte size is enlarged, followed by decompensatory hypertrophy or myocardial weakening. In our current study we are attempting to understand the temporal resolution of the development of compensatory hypertrophy through the analysis of underlying functional and molecular changes. Through the use of a novel multicellular preparation culture technique we are able to induce load dependent hypertrophy on isolated muscle preparations from rabbit myocardium. Recently, we have shown that at high load there is an increase in developed force at 24 hours in culture followed by a drastic decline in force and eventually failure at 48 hours. However, load and time dependent fluctuations in other kinetic functions, including relaxation time and time to peak force, do not occur. We have also seen increases in expression of troponin I (TnI), myosin heavy chain (MHC), the sodium-calcium exchanger (NCX), and troponin T (TnT) at 24 hours in culture. This data taken together suggests the induction of compensatory hypertrophy at 24 hours in our culture system. The use of this novel multi-cellular culture preparation technique will allow us to quantify gradual changes in kinetic function and changes in protein expression at multiple time points validating this technique for future cardiac studies.

NEUROENDOCRINE PEPTIDES NEUROPEPTIDE-Y (NPY) AND PEPTIDE-YY (PYY) SUPPRESS Cl^- SECRETION AND K^+ SECRETION IN GUINEA PIG DISTAL COLON THROUGH ACTION AT Y2-RECEPTORS

Susan Halm, Jin Zhang and Dan Halm
Department of Neuroscience, Cell Biology & Physiology, Wright State University Boonshoft School of Medicine, Dayton OH 45435 (dan.halm@wright.edu)

Electrogenic Cl^- and K^+ secretion in isolated mucosa from guinea pig distal colon measured as short-circuit current (I_{sc}) and transepithelial conductance (G_t) were stimulated by epinephrine (epi), prostaglandin-E_2 (PGE_2) and carbachol (CCh). neuropeptide-Y (NPY) and peptide-YY (PYY) inhibited by 60% Cl^- secretion activated by either PGE_2 or PGE_2+CCh with EC_{50}'s of 16nM and 6nM, respectively. Neither peptide markedly inhibited the transient component of the PGE_2+CCh response. Immunoreactivity (IR) for NPY was present in enteric ganglia and in proximity with crypts. Basolateral membranes of colonic crypt and surface epithelial cells had distinct IR for neuropeptide-Y receptors Y1 and Y2. Receptor expression was supported further by immuno-blot showing bands at molecular weights consistent with monomer and oligomer. Sub-type selective antagonists BVD10 (Y1) and BIIE0246 (Y2) indicated that secretory suppression occurred through Y2 receptors. The Y2 selective peptide PYY(3-36) also suppressed Cl^- secretion with a EC_{50} of 9nM. BIIE0246 addition increased I_{sc} and G_t during PGE_2 or PGE_2+CCh activation consistent with the presence of in vitro released PYY or NPY. PYY addition increased the epi EC_{50} to 18nM compared with BIIE0246 treated tissues, 4nM. Thus, PYY or NPY suppressed Cl^- secretory capacity and desensitized the adrenergic K^+ secretory response, providing a negative feedback for secretory activation. [supported by NIH, DK65845]

CONFIRMATION OF A NEOINTIMAL HYPERPLASIA QUANTITATIVE TRAIT LOCI ON CHROMOSOME 3 RELATED TO VASOREACTIVITY.

Andrea L. Nestor-Kalinoski[1,3], Ramona S. Ramdath[1,3], Kay M. Langenderfer[1,3], Saad Sikanderkhel[1,3], Sarah DeRaedt[1,3], Marlene Welch[1,3], George T. Cicila[2] and David C. Allison[1-3].

Departments of [1]Surgery, [2]Physiology, Pharmacology, Metabolism and Cardiovascular Diseases and the [3]University of Toledo Advanced Microscopy & Imaging Center, University of Toledo, College of Medicine, 3035 Arlington Avenue, Toledo, Ohio 43614 Corresponding Author: andrea.kalinoski@utoledo.edu

Restenosis following cardiovascular interventions in the vascular system occurs in upwards of 37% of patients undergoing procedures such as arterial grafts, stents, and balloon angioplasties due to development of neointimal hyperplasia (NIH).
To identify mechanisms and/or gene(s) underlying the heritable differences that contribute to vascular restenosis after injury, we performed a genome scan on an intercross population derived from the Spontaneously Hypertensive Rat (SHR) and Brown Norway (BN) rat strains. Linkage analysis identified two quantitative trait loci (QTL) for NIH on chromosomes 3 (RNO3) and 6 (RNO6) with LOD scores of 2.5 and 2.2 respectively. Congenic strains were developed by introgressing the QTL-containing regions from the BN (donor strain) onto the SHR genetic background (SHR.BN3 and SHR.BN6) and phenotyped for NIH. The %NIH in the SHR.BN3 congenic was 28.3%, significantly higher ($p = 0.001$) compared to the parental SHR (9.6%) strain. However, the presence of an NIH QTL on RNO6 was not confirmed by congenic analysis. In a separate experiment, a "double" congenic strain (SHR.BN3+6) was generated to determine if there was an additive effect in the QTLs. The "double" congenic strain demonstrated 23.8% neointimal growth, which was significantly higher compared to the parental SHR, but did not demonstrate an additive increase in NIH compared to the SHR.BN3 congenic strain. Further, we evaluated the vasoreactivity of vessels isolated from the parental and congenic strains with several potent vasoactive agents (PE, 5HT and $PGF_{2\alpha}$). Interestingly, we observed a significant difference ($p < 0.01$) in the vasoconstrictive response of SHR.BN3 congenic rats compared to the BN, SHR and SHR.BN6 strains with both 5HT and $PGF_{2\alpha}$. This finding of a 43 Mb NIH QTL-containing region on RNO3 will allow the further dissection of the region to identify candidate gene(s) controlling NIH and may facilitate the development of new therapies to prevent this complication.

THE POINT MUTATION CASQ2^{D307H} DOES NOT AFFECT CALSEQUESTRIN EXPRESSION AND TARGETING TO THE jSR.

Anuradha Kalyanasundaram and Muthu Periasamy
Department of Physiology and Cell Biology, Ohio State University. Corresponding author: kalyanasundaram.2@osu.edu

Calsequestrin (CASQ2) is the major calcium binding protein located in the junctional sarcoplasmic reticulum (jSR). Recently, several mutations have been reported in human CASQ2 and have been linked to Catecholamine induced Polymorphic Ventricular Tachycardia (CPVT). Of these mutations, CASQ2^{D307H}, a point mutation that changes the negative aspartate (D) to a positive histidine (H) has been shown to affect SR calcium cycling thereby inducing arrhythmia. This mutation was proposed to affect protein expression and stability resulting in a CASQ2 null phenotype. To better define how the point mutation affects CASQ2 structure /function in the heart, we generated a mouse model that expresses the mutant protein in a CASQ2 null background. In this study we demonstrate that the mutant protein can be stably expressed. We additionally show that it is properly targeted and localized to the jSR. It is also conceivable that expression of mutant CASQ2 can induce ER stress, which could cause a disease phenotype. Therefore, we investigated if CASQ2^{D307H} expression induces an ER stress response. We found that the expression of ER stress markers GRP78, GRP94 and ER chaperone protein Calreticulin are not altered in hearts expressing CASQ2^{D307H} mutant protein. These findings indicate that the D307H mutation does not affect the expression, stability and trafficking of CASQ2 but could potentially affect protein/protein interaction among the jSR proteins including triadin, junctin and ryanodine receptor.

REGULATION OF Na/K-ATPase TRAFFICKING BY CHOLESTEROL

Xin Li,[1] Yiliang Chen,[1] Zijian Xie[1]
[1]*Department of Physiology and Pharmacology, College of Medicine, University of Toledo, Toledo, Ohio 43614-2598*
Corresponding Author: *xli12@utoledo.edu*

We have shown that graded knockdown of the Na/K-ATPase increases endocytosis and mobility of caveolin-1(Cav1), resulting in decreases in the plasma membrane pool of Cav1 (Cai, et al, J Cell Biol. 2008, 182:1153-69). Moreover, we have found that the Na/K-ATPase knockdown also reduces the amount of plasma membrane cholesterol. Because depletion of plasma membrane cholesterol increases caveolin-1 endocytosis, we reckon that cholesterol-mediated regulation of caveolin-1 trafficking may involve changes in the expression and trafficking of Na/K-ATPase. Using a combination of Western blot and confocal imaging analyses we demonstrate that depletion of plasma membrane cholesterol by either addition of MβCD or maintaining cells in lipoprotein-deficient medium was sufficient to stimulate the endocytosis and consequently reduce the cellular amount of Na/K-ATPase. Consistently, addition of U18666A, a compound that reduces plasma membrane pool of cholesterol by disrupting cholesterol trafficking, produces the same phenotype in cultured cells. Taken together, our new findings demonstrate a novel function of cholesterol in control of Na/K-ATPase expression and distribution. They also suggest that the effect of cholesterol on caveolin-1 trafficking may involve reduction of the plasma membrane pool of Na/K-ATPase. Supported by NIH grants (HL-36573, HL-67963, and GM-78565)

NaKtide, Na/K-ATPase-DERIVED PEPTIDE Src INHIBITOR, ANTAGONIZES OUABAIN-ACTIVATED SIGNAL TRANSDUCTION IN CULTURED CELLS

Zhichaun Li, Ting Cai, Lijun Liu, Jiang Tian, Joe X. Xie, Xiaochen Zhao and Zi-Jian Xie
Department of Physiology and Pharmacology
University of Toledo College of Medicine, 3035 Arlington Av., Toledo, OH 43614
zhichuan.li@utoledo.edu

Many studies have demonstrated nanomolar concentration of ouabain activates Src through Na/K-ATPase/Src receptor complex, and then consequently induce activation of several signaling pathways, including activation of MAPK pathway, mitochondrial release of reactive oxygen species (ROS), and transient release of intracellular Ca^{2+}. The formation of Na/K-ATPase/Src complex involves the interaction between the third cytosolic domain (CD3) of α1 and kinase domain of Src. This interaction functionally keeps Src in inactive state, while ouabain binding will release the kinase domain and lead to Src activation. However, it is still unknown how the CD3 inhibits Src. Here, we further identified the binding motifs within the CD3. First, GST pulldown assay showed a direct interaction between the N-terminus (ND1) of N domain (nucleotide-binding domain) of α1 and Src. FRET analysis indicated ND1 was able to associate with Na/K-ATPase/Src complex in LLC-PK1 cells. Functionally, ND1 significantly inhibited Src activity in test tubes as well as LLC-PK1 cells. Second, a 20-residue peptide, denoted as NaKTide, encompassing amino acid residues from ND1, was capable to inhibit Src in a dose-dependent manner. Accordingly, cell-permeable NaKTide consisting of NaKTide coupled to GRKKRRQRRRPPQ significantly inhibited Src with IC_{50} around 4 nM, while no inhibitory effect on PKC activity up to 10 μM. Consistently, permeable NaKTide inhibited basal Src as well as ERK activity in PY-17 cells where these kinases activities were elevated specifically due to knockdown of Na/K-ATPase. Finally, preincubation with permeable NaKTide abrogated ouabain-induced activation of Src and then blocked MAPK signaling pathway in both LLC-PK1 and neonatal cardiac myocytes. These new findings indicate that NaKTide may act as a potential ouabain antagonist. Moreover, NaKTide makes it possible for us to further address the function role of digitalis-induced signaling through Na/K-ATPase.

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SKELETAL MUSCLE ENERGETICS FOLLOWING COLD ACCLIMATION IN A BROWN ADIPOSE TISSUE DEFICIENT MOUSE.

MINEO, PM*; ROBERTS, ME; SCHAEFFER, PJ
Miami University Department of Zoology (mineopm@muohio.edu)

We are interested in the plasticity of the skeletal muscle metabolic phenotype during cold acclimation in the UCP-dta mouse, and how metabolic acclimation to cold might influence obesity. UCP-dta mice, a transgenic line lacking brown adipose tissue (BAT), become obese when housed at standard animal facility temperatures. In addition, the development of obesity can be accelerated when UCP-dta mice are fed a high fat diet. When presented with an acute cold challenge, summit VO_2 in UCP-dta mice fed standard chow were significantly lower than wild type controls. When fed a high fat diet alone, there was no additional effect on summit VO_2. These results show that the lack of functional BAT in these mice greatly reduces their ability to produce heat during an acute cold challenge as shivering in skeletal muscle is not able to compensate for the absence of BAT. Future experiments will investigate the effects of cold acclimation on the aerobic capacity of skeletal muscle, and how the increased activity due to shivering affects muscle energetics.

THE POSITIVE FORCE-FREQUENCY RELATIONSHIP IS MAINTAINED IN ABSENCE OF SARCOPLASMIC RETICULUM FUNCTION IN RABBIT, BUT NOT IN RAT MYOCARDIUM

Michelle M. Monasky and Paul M. L. Janssen*

Department of Physiology and Cell Biology, College of Medicine, The Ohio State University, 1645 Neil Avenue, Columbus, OH 43210 USA.
**Correspondence to janssen.10@osu.edu*

Myocardial calcium handling differs between species, mainly in the relative contribution between the sources for activator calcium. To investigate the role of the myofilaments and intracellular calcium decline in governing the relaxation phase of cardiac muscle, and to elucidate additional determinants of relaxation other than the sarcoplasmic reticulum (SR) at various frequencies within the *in vivo* range, the present study was performed by altering the calcium handling in rat and rabbit. Trabeculae at optimal preload and at 37 ˚C were iontophoretically loaded with bis-fura-2 to monitor cytoplasmic calcium levels before being subjected to ryanodine and cyclopiazonic acid to inhibit SR function. Simultaneous force and $[Ca^{2+}]_i$ measurements were obtained at 1-4 Hz in rabbit and at 4-8 Hz in rat before and after SR inhibition. Inhibition of the SR resulted in increased diastolic calcium from 72 ± 7 nM to 101 ± 15 nM in rabbit at 1 Hz and from 99 ± 3 nM to 244 ± 23 nM in rat at 4 Hz. Peak calcium levels increased as well, from 287 ± 55 nM to 645 ± 136 nM in rabbit at 1 Hz and from 297 ± 39 nM to 432 ± 57 nM in rat at 4 Hz. Developed force increased with frequency in rabbit after inhibition of SR function, from 6.7 ± 1.4 mN/mm^2 at 1 Hz to 16.3 ± 3.8 mN/mm^2 at 4 Hz. However, developed force decreased with frequency in rat after inhibition of SR function, from 6.3 ± 2.3 mN/mm^2 at 4 Hz to 4.0 ± 0.6 mN/mm^2 at 8 Hz, despite the fact that both species normally exhibit a positive force-frequency relationship. Calcium transient amplitude decreased in rat, but increased in rabbit after SR inhibition. Time to peak tension, time from peak tension to 50% relaxation, time to peak calcium, and time from peak calcium to 50% calcium decline were all prolonged. Results suggest that L-type calcium channel current is responsible for increases in calcium with increasing frequency, and that the SR amplifies this effect in response to increased L-type current. The response of the myofilaments to alterations in calcium handling plays a critical role in the final determination of force, and may differ between species. These results imply the balance between force relaxation and calcium decline is significantly different in larger mammals, necessitating a critical re-evaluation of how myocardial relaxation is governed, specifically regarding frequency-dependent activation.

MICE WITH NULL MUTATION OF CEACAM1 EXHIBIT METABOLIC SYNDROME WITH CARDIAC HYPERTROPY

Eric E. Morgan[1], Kelly J. Ledford[1], Anthony M. DeAngelis[1], Payal Patel[1], Sonia M. Najjar[1], Samer Khouri[2], and Sandrine V Pierre[1]
Departments of [1]Physiology and Pharmacology and [2]Medicine, University of Toledo College of Medicine, 3035 Arlington Avenue, Toledo, Ohio 43614 Corresponding author: eric.morgan@utoledo.edu

Understanding the interaction between the metabolic syndrome, a major risk factor for cardiovascular disease, and cardiovascular dysfunction has been complicated by the lack of animal models that recapitulate the pathophysiology of the metabolic syndrome in humans. Because the carcinoembryonic antigen-related cell adhesion molecule 1 (*Ceacam1*) regulates insulin and lipid metabolism in liver, we hypothesized that null mutation of *Ceacam1* (*Cc1*$^{-/-}$) results in a phenotype that mimicks metabolic syndrome and displays cardiovascular abnormalities. In the present study, we characterized the metabolic and cardiovascular phenotypes of six month-old male Cc1$^{-/-}$ mice.

As Table 1 reveals, *Cc1*$^{-/-}$ mice developed visceral obesity and elevated fasting plasma free fatty acids (FFA). Consistent with a role for *Ceacam1* in promoting insulin clearance in liver, the mice also developed hyperinsulinemia and insulin resistance. Normal fasting glucose levels reflect normal β-cell function and increased compensatory insulin secretion. *Cc1*$^{-/-}$ mice exhibited higher systolic blood pressure as revealed by tail-cuff measurements. Finally, echocardiograpic measurements of left ventricular end-diastolic diameter (LVDd), septal (SW) and posterior (PW) wall thicknesses revealed an increased relative wall thickness (RWT; calculated as SW+PW/LVDd). No differences in systolic function were observed (data not shown).

Table 1. Metabolic and Cardiovascular Phenotypes

	Cc1 $^{+/+}$	(n)	*Cc1* $^{-/-}$	(n)
Body Weight (g)	27.24 ± 0.31	(65)	30.56 ± 0.30	(60) ***
Visceral adiposity (% of Body Weight)	1.9 ± 0.1	(8)	3.3 ± 0.3	(9) ***
Fasting Blood Glucose (mg/dl)	103.24 ± 3.0	(21)	98.53 ± 2.26	(20) *NS*
Fasting Insulin (pM)	56.97 ± 6.73	(8)	171.41 ± 37.47	(11) *
FFA (mmol/L)	0.77 ± 0.08	(9)	1.05 ± 0.04	(12) **
Systolic blood pressure (mm Hg)	113.0 ± 4.2	(13)	169.0 ± 7.5	(14)***
RWT	0.42 ± 0.03	(13)	0.59 ± 0.06	(14)*

NS. Non Significant; * $p<0.05$; ** $p<0.001$; and *** $p< 0.0001$ *vs. Cc1* $^{+/+}$

We conclude that null mutation of *Ceacam1* mimics metabolic syndrome, including marked elevation in systolic blood pressure and left ventricular hypertrophy with preserved systolic function.

PPARγ2-MEDIATED PROTEOLYTIC DEGRADATION OF β-CATENIN DETERMINES AN ANTI-OSTEOBLASTIC EFFECT OF ANTI-DIABETIC TZDS

S. Rahman, P. Czernik, B. Lecka-Czernik
University of Toledo College of Medicine
Corresponding author: sima.rahman@utoledo.edu

The transcription factor PPARγ2 is a key regulator of marrow mesenchymal stem cells (MSCs) differentiation. It positively regulates adipocyte and suppresses osteoblast differentiation. Anti-diabetic drugs thiazolidinediones (TZDs), which specifically activate PPARγ protein, upregulate fat production in the bone marrow and cause bone loss in animals and humans. In order to understand the mechanisms of TZD-induced bone loss, we performed microarray analysis of gene expression changes in a cellular model of PPARγ2-controled MSC differentiation, U-33/γ2 cells. Among the early responders to R were the proteins involved in Wnt signaling pathway, which is essential for MSC differentiation toward osteoblast and bone formation. The expression of WISP-1 and Tle3 was significantly altered within 2 h post treatment followed by the suppressive effect on the expression of multiple members of this pathway including Fzd receptors, Dkk1, Sfrp1, and Wif1 modulators, and Tcf3 and Tcf4 transcriptional effectors. Cellular silencing of WISP-1 and Tle3 using siRNA suggested that they are not the major mediators of R-induced suppression of osteoblast phenotype. Therefore, we tested whether R affects activity of β-catenin, a key mediator of Wnt signaling. We found that R-activated PPARγ2 induced proteolytic degradation of more than 90% of the unbound or active form of β-catenin as early as within 1 hr post treatment, not affecting a pool of protein bound (inactive) β-catenin. Consistent with this, R suppressed transcriptional activity of β-catenin, measured by the activity of TOP-FLASH construct in a luciferase gene reporter assay. Moreover, R suppressed alkaline phosphatase activity even in the presence of LiCl, which stabilizes an active form of β-catenin. To test whether β-catenin degradation is responsible for R anti-osteoblastic effects, we modified PPARγ2 protein domains responsible for β-catenin degradation. Using transiently transfected marrow MSC with mutated PPARγ2 constructs, we are currently testing the hypothesis that a lack of PPARγ2 proteolytic activity for β-catenin protects Wnt pathway gene expression and osteoblast phenotype against the negative effect of anti-diabetic TZDs

MENIN IS INVOLVED IN CEACAM 1 MODULATION OF THE METABOLIC PATHWAY ASSOCIATED WITH OBESITY AND TYPE 2 DIABETES

Sadeesh Ramakrishnan, Sonia Najjar and Edith Mensah-Osman
Center for Endocrine and Diabetes Research; Department of Physiology & Pharmacology. University of Toledo College of Medicine, Toledo, OH, USA. Corresponding author: Sadeesh.Ramakrishnan@utoledo.edu

Menin is a tumor suppressor protein, lost in the Men 1 syndrome, and associated with neuroendocrine tumors, with an increased prevalence of diabetes. Ceacam1 is implicated in the development of type 2 diabetes and visceral obesity. Since Ceacam1 is central to the disorders associated with Men 1 syndrome we asked the question; does menin regulate ceacam1.

We utilized 3 transgenic mouse models of menin, Ceacam 1 and Pten to discern the association of menin and Ceacam 1 on cholesterol regulation, fatty acid synthesis, visceral obesity and the onset of type 2 diabetes. Western blot analysis was used for protein determination, and qRT-PCR for RNA levels. Double staining by immunoflourescence or immunohistochemistry was used to determine the localization of menin and ceacam 1.

Preliminary results show that levels of (i) CEACAM1 are reduced in the liver and intestine of transgenic mice that have one allele of the menin gene deleted in the whole body, whereas menin levels are not altered in total body loss of ceacam 1. (ii) Loss of menin results in increased visceral obesity, fatty liver with limited inflammation. (iii) Fasting and re-feeding regulates the overall expression levels of menin in the liver, consistent with insulin surges. (iv) menin and ceacam1 are co-expressed in unique cells in the gut and in close proximity within the liver and pancreatic cells. (v) Menin levels are reduced in livers of obese subjects compared to lean counterparts.

We conclude that loss of menin in the liver is associated with metabolic changes leading to the development of visceral obesity, fatty liver disease and type 2 diabetes.

THE EFFECT OF EXERCISE IN PREVENTING OBESITY AND METABOLIC DISEASE IN A MOUSE MODEL

Roberts, Maureen E.; Mineo, Patrick; Schaeffer, Dr. Paul
Miami University (robertm6@muohio.edu)

This project is an investigation of the impact of exercise on insulin sensitivity of skeletal muscle in mice. Mice were either wild-type or UCP-dta transgenic mice (which lack brown adipose tissue). Diet consisted of either standard chow or high-fat (45% of calories). Mice were either sedentary or had running wheels available for voluntary exercise for 6 weeks. Body weights were recorded every other day to track weight gain. The running patterns of exercising mice were recorded in order to calculate average daily activity. In order to determine the role of these factors in insulin sensitivity, we performed Glucose Tolerance Tests (GTT) on 12 week old mice. As previously reported, transgenic mice fed a high fat diet developed obesity. All mice with access to running wheels ran >5 hours each night. In mice fed standard chow, there was no significant effect of genotype or exercise on results of GTTs. In mice fed high fat chow, all groups had impaired responses in GTTs. Although sample size is low, transgenic mice appeared to have a more impaired GTT response. Voluntary exercise did not appear to improve the response to GTTs in the high fat fed mice. Replication will increase sample size to validate the significance of these results and future experiments will investigate the effects of higher intensity treadmill exercise on insulin sensitivity in these mice.

THE MECHANISM OF DEFECTIVE ER-TO-GOLGI TRAFFICKING OF ΔF508 CFTR.

Gargi Roy and Dr. Xiaodong Wang
University of Toledo Corresponding author: Gargi.Roy@utoledo.edu

Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) is a cell surface chloride channel consisting of two membrane spanning domains, two nucleotide binding domains (NBDs) and a regulatory domain. A deletion of phenylalanine at position 508 (ΔF508) is present in over 90% of the CF patients. This results in the retention of the immature protein in the endoplasmic reticulum (ER) and its subsequent degradation through ubiquitin-proteosome pathway. The precise mechanism of the defective ER-to-Golgi export of ΔF508 CFTR is not known. A di-acidic ER exit code (DAD) has been identified within NBD1. Disruption of this motif leads to the defective coupling of CFTR to the COPII machinery and the subsequent impaired export of CFTR from the ER. F508 also resides in NBD1, the deletion of which reduced its coupling to the COPII machinery and abolishes its exit from the ER. The functional connection between ΔF508 and the exit code DAD is currently missing. Both export and retention signals have been identified within CFTR. We performed a systematic analysis of the potential ER-to-Golgi sorting signals within CFTR, and specifically examined the functional relationship between different sorting signals in the context of ΔF508 CFTR. We found that the temperature-dependent export of ΔF508 relies on the presence of the di-acidic code within the NBD1, suggesting that this code has a functional role in the temperature-dependent rescue of ΔF508 CFTR. ΔF508 CFTR rescue by the simultaneous disruption of two of the arginine-framed triprptide (AFT) is also dependent upon the di-acidic code. Of the two AFT, R555K alone is sufficient to mediate ΔF508 CFTR rescue. In situ limited proteolysis revealed conformational changes in both TMD1 and NBD2, which are corrected by R555K. These results indicate ΔF508 CFTR has impaired domain conformation that negatively impacts the functional utilization of the di-acidic motif. Second site mutation such as R555K rescues ΔF508 CFTR through correcting its defective conformation.

Hsp105 REVEALS DISTINCT CONFORMATIONAL MATURATION PATHWAYS FOR WILD-TYPE AND ΔF508 CFTR.

Saxena, A.*; Chalfin, E.M.; and Wang, X.
University of Toledo. Corresponding author: Anita.Saxena@utoledo.edu

ΔF508 CFTR, the most prevalent mutant in patients with cystic fibrosis, fails to exit the endoplasmic reticulum (ER) due to defective conformational maturation. However, little is known about the folding pathway of wild-type CFTR prior to its exit from the ER, and how the ΔF508 mutation prevents CFTR forward folding. The Hsp70 family of molecular chaperones plays important roles in the protein quality control process within the ER. Hsp105, a high-molecular-weight member of the Hsp70 family was recently shown to display nucleotide exchange activity for Hsp70 in vitro. Furthermore, Hsp105 was identified as a component of a CFTR-associated multiple protein complex using a global proteomic approach (Cell 127: 803-815, 2006). In an attempt to explore the role of Hsp105 in CFTR conformational maturation in the ER, we over-expressed the co-chaperone in HEK293 cells and quantitatively analyzed its effect on the maturation and degradation of CFTR. Consistent with its role as a nucleotide exchange factor for Hsp70s, over-expression of Hsp105 inhibits the ER export of wild-type CFTR and promotes its degradation. However, in striking contrast, over-expressing Hsp105 stabilizes ΔF508 CFTR and promotes its ER export both at physiological as well as at reduced temperature. The apparently opposite effects of Hsp105 on wild-type and ΔF508 CFTR maturation and quality control, suggests distinct conformational maturation pathways for the two CFTR molecules, and reveals a specific role for Hsp105 in regulating ΔF508 CFTR refolding. Such conclusion is reinforced by RNAi experiments. RNAi-mediated down-regulation of Hsp105 expression reduced the export of ΔF508 CFTR at reduced temperature. Further studies are necessary to achieve a better understanding of the machinery, pathway and mechanism of ΔF508 CFTR conformational maturation at reduced as well as physiological temperatures, and this in turn will provide critical insights and key factors that are of potential value to the rescue of the trafficking defect of ΔF508 CFTR.

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SEASONAL ACCLIMATION THROUGH CHANGES IN ENERGETICS IN NORTHERN CARDINALS *(Cardinalis cardinalis)*

Carrie Sgueo, Dominique Wagner, Patrick Walsh, Brett Aiello, and Dr. Paul Schaeffer
Miami University of Oxford, OH. Corresponding author: sgueoc@muohio.edu

Avian species are faced with varying seasonal challenges that must be met to ensure survival, particularly non-migrant species that undergo thermoregulatory challenges during harsh winter conditions. The summer is also energetically expensive due to breeding and parental care. In order to respond to environmental variation throughout a given year, physiological changes in energy use may be occurring at both organismal and cellular levels; therefore seasonal acclimation may be accomplished by phenotypic plasticity of energetic capacity. We will measure daily energy expenditure using heart rate transmitters, maximum metabolic capacity, and muscle oxidative capacity in the Northern Cardinal (*Cardinalis cardinalis*) in winter (December to January) and breeding season (May to June), in order to test the hypothesis that phenotypic plasticity of energetics is an important part of seasonal acclimation, and maybe determine which (if either) season is more costly.

ANGIOTENSIN II-INDUCED ERK1/2 ACTIVATION IS MEDIATED BY PKCδ AND INTRACELLULAR CALCIUM IN CARDIAC FIBROBLASTS

Patricia E. Shamhart[1,2], Erik R. Olson[1,2], Daniel J. Luther[1,2], Jennifer E. Naugle[1,2], , Kathleen J. Doane[1,2], and J. Gary Meszaros[1,2]
[1]Department of Integrated Medical Sciences, Northeastern Ohio Universities College of Medicine, Rootstown, OH: [2]Graduate Program, School of Biomedical Sciences, Kent State University, Kent, OH Corresponding author: jgmeszar@neoucom.edu

Activated cardiac fibroblasts (CFs) proliferate, differentiate, and migrate to regulate cardiac remodeling. ANG II activates extracellular signal-regulated kinase (ERK) 1/2 to induce proliferation, but the signaling pathways leading to ERK 1/2 activation have not been elucidated in CFs. The goal of the current study was to identify the intracellular mediators of ANG II-induced ERK 1/2 activation in adult rat CFs. We determined that ANG II does not transactivate the epidermal growth factor receptor (EGFR) in adult CFs, since pretreatment with AG 1478 did not inhibit [^{3}H]-thymidine incorporation or ERK 1/2 activation. We found that ANG II-induced ERK 1/2 phosphorylation is inhibited by simultaneous chelation of cytosolic Ca^{2+} and downregulation of PKC by phorbol ester or by the specific PKCδ inhibitor rottlerin as well as PKCδ siRNA, but not by inhibition of each agent alone. We next investigated the role of specific PKC isoforms in migration of the activated CFs using in vitro wound healing assays. Through pharmacological blockade we determined that inhibition of PKCδ slows migration of CFs and inhibition of PKCα also decreases the rate migration but to a lesser extent than blockade of PKCδ. These data reveal that PKCδ is a key mediator in both ANG II-induced proliferation with concurrent Ca^{2+} chelation and migration of CFs.

REGULATION OF SYNAPTIC NR2B SUBUNIT-CONTAINING NMDA RECEPTORS: ONE COMPONENT OF GLUTAMATERGIC PLASTICITY IN BENZODIAZEPINE WITHDRAWAL-ANXIETY

G. Shen and E. I. Tietz
Department of Physiology and Pharmacology, Neuroscience and Neurological Disorders Track, University of Toledo Health Science Campus, College of Medicine, Toledo, OH 43614 Corresponding Author: guofu.shen@utoledo.edu

Excitatory glutamatergic neurotransmission is modified in rats withdrawn from 1-week oral administration of the benzodiazepine, flurazepam (FZP). Insertion of GluR1-containing AMPARs into hippocampal CA1 neuron synapses results in a progressive increase in AMPAR synaptic currents from day 1 to day 2 of withdrawal. Conversely, a reduction in NMDAR function is reflected in a 50% decrease in NMDA-elicited whole-cell current in dissociated CA1 neurons and in NMDAR-mediated evoked EPSC in hippocampal slices, attributable to a decrease in NR2B subunit mRNA and protein, measured *in situ*. Enhanced AMPAR function, linked to the appearance of withdrawal-anxiety in day 1, can negatively modulate NMDAR function in these neurons on day 2. NMDAR EPSCs (V_H=+40mV) evoked by Schaffer collateral stimulation were recorded in hippocampal slices from 2-day FZP withdrawn rats in the presence of DNQX, picrotoxin and CGP35348. As before, the amplitude of half-maximal eEPSCs decreased in the FZP-withdrawn group (CON: 491±87 pA vs. FZP: 247±43 pA, $p<0.05$). Ifenprodil, a NR2B specific antagonist, abolished the difference in eEPSC amplitude between these two groups. The peak ifenprodil-sensitive component was decreased in FZP-withdrawn neurons (CON: 47.9±8.5% vs. FZP: 9.5±8.9%, $p<0.05$) without a change in single exponential decay (CON: A1=290 pA, tau=150 ms vs. FZP: A1=126 pA, tau=140 ms). To evaluate whether NR2B subunit is changed in the CA1 region, CA1 minislices were collected from 2-day withdrawn rats and fractionated into cytosolic (S2), membrane (P2) and postsynaptic density (PSD)-enriched fractions (P3). Immunoblot revealed a significant decrease of synaptic NR2B subunit expression levels (CON: 100±5.3% vs. FZP: 75.6±7.6%, $p<0.05$), along with moderate decrease of NR1 subunit (CON 100±2.4% vs FZP: 85.0±3.9%, $p<0.05$). Levels of the CaMKII substrate, phopho-Ser1303 NR2B decreased proportionately in the same fraction. Samples from 1-day withdrawn rats were also evaluated for changes in the levels of the same NMDA and AMPA receptor subunits. Unlike in previous studies in 2-day FZP-withdrawn rats in which both phospho-Ser831 and GluR1 subunits were elevated, only GluR1 subunit levels increased significantly (CON 100±2.4% vs FZP: 156±12%, $p<0.05$) in the P3 fraction, consistent with GluR1-containing AMPAR insertion prior to Ser831 phosphorylation. Collectively, these findings suggest that a reduction in NR2B subunit-containing NMDARs may serve as compensatory mechanism to mitigate enhanced AMPAR glutamatergic strength during FZP withdrawal to alleviate the associated benzodiazepine withdrawal symptoms.

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TNFα INDUCED VCAM-1 EXPRESSION AND MONOCYTE ADHESION REQUIRES TRPC3 MEDIATED CONSTIUTIVE Ca^{2+} INFLUX IN CORONARY ARTERY ENDOTHELIAL CELLS

Kathryn Smedlund and Guillermo Vazquez
Department of Physiology & Pharmacology, University of Toledo Health Science Campus
Guillermo.Vazquez@utoledo.edu

Tumor necrosis factor-alpha (TNFα) is a strong regulator of the inflammatory response of vascular endothelium to pro-atherogenic stimuli. In a recent study, we have shown that TNFα contributes to the upregulation of vascular cell adhesion molecule-1 (VCAM-1) expression in human coronary artery endothelial cells (HCAECs) (Smedlund and Vazquez, 2008, Arterioscler Thromb Vasc Biol, in press). VCAM-1 is a critical mediator in monocyte recruitment to the endothelium, one of the earliest events in the development of atherosclerotic lesions. Our recent findings indicate that in HCAECs TNF-α promotes VCAM-1 expression and function through a mechanism that requires Ca^{2+} influx through plasma membrane Ca^{2+} channels. Omission of Ca^{2+} in the extracellular bath or pre-treatment of the cells with Ca^{2+} channel blockers significantly reduced TNFα stimulated VCAM-1 expression and monocyte adhesion. Knockdown of the channel forming protein TRPC3 by means of siRNA also markedly impaired VCAM-1 expression and function. In HCAECs native TRPC3 channels mediate both regulated (receptor-stimulated) and non-regulated (constitutive) Ca^{2+} influx. Using real-time fluorescence to monitor changes in intracellular Ca^{2+} levels, we found that TNFα did not induce Ca^{2+} release or entry in HCAECs, nor activation of TRPC3 in an overexpression system. Notably, in HCAECs TNFα-induced VCAM-1 was accompanied by an increase in TRPC3 expression and constitutive cation influx, indicative of an increase in the population of functional channels in the plasma membrane. Altogether, our findings suggest that in coronary endothelium the mechanism underlying TNFα induced VCAM-1 expression and monocyte adhesion partially depends on TRPC3-mediated constitutive Ca^{2+} entry rather than on regulated channel activity. We speculate that increased expression of TRPC3, a channel bestowed with high constitutive activity, in response to pro-atherogenic stimuli, may represent a contributing factor to the pathogenesis of coronary artery disease.

ROLE OF Ca^{2+} INFLUX IN ACUTE ATP-DEPENDENT REGULATION OF eNOS IN AORTIC ENDOTHELIAL CELLS

Faten Smiley and Guillermo Vazquez
Department of Physiology & Pharmacology, University of Toledo Health Science
Guillermo.Vazquez@utoledo.edu

Atherosclerosis is the leading cause of death in western societies and remains the major vascular complication of obesity, metabolic syndrome and diabetes. At the cellular/molecular level the disease results from a complex interplay between mechanisms of the vascular endothelium (e.g., endothelial dysfunction, inflammation) driven by a variety of genetic and environmental risk factors. Regardless of the mechanism, decreased production and/or availability of endothelial nitric oxide (NO) is a common finding. For instance, reduced NO production and/or dysregulation of eNOS activity promote recruitment of inflammatory cells and oxidative stress. Whereas the importance of phosphorylation and Ca^{2+} influx in regulation of eNOS activity is well recognized, they have been envisioned for the most part, as independent regulatory limbs, and information on how they crosstalk to achieve a common goal is controversial. Canonical Transient Receptor Potential (TRPC) channels (TRPC1-TRPC7) are among the most important Ca^{2+}-permeable cation channels in vascular endothelium and it is becoming evident that they have a central role in the pathogenesis of cardiovascular disease. Our lab identified TRPC3 as an obligatory component of the endothelial inflammatory response that occurs early in atherogenesis. In human coronary (HCAEC) and aortic (HAEC) endothelial cells TRPC3 expression is upregulated by pro-atherogenic stimuli (e.g., ATP, TNFα) and in HCAEC TRPC3-mediated Ca^{2+} influx is required for monocyte adhesion, a critical event in all stages of atherosclerotic lesion. We here examined the contribution of Ca^{2+} influx to regulation of eNOS by pro-inflammatory stimuli to set the ground for further studies aimed at exploring the role of TRPC proteins in endothelial dysfunction and inflammation within the context of NO generation. We used HAEC cells as an in vitro endothelial model and immunochemical and Fura-2 based real-time fluorescence techniques to determine the activation of eNOS under different experimental conditions. ATP-dependent stimulation of HAEC resulted in a biphasic Ca^{2+} response (Ca^{2+} release and Ca^{2+} influx) which was accompanied by acute (5-15 min) and transient activation of eNOS (indirectly assessed from the extent of phosphorylation of Ser-1177). Notably, this effect was significantly reduced when Ca^{2+} was omitted from the extracellular bath, or blocking ATP-induced Ca^{2+} influx by pre-treating the cells with verapamil or SKF96365, two channel blockers known to markedly reduce activity of most TRPC channels. These findings support the notion that in HAEC native Ca^{2+}-permeable channels contribute to Ca^{2+} influx following stimulation of purinergic receptors, and that Ca^{2+} influx is fundamental within the signaling underlying eNOS phosphorylation. Further studies are required to determine which TRPC isoforms contribute to ATP-induced Ca^{2+}-influx and eNOS phosphorylation in these cells, as well as the impact of upregulated channel expression (e.g., TRPC3) on this process under pro-atherogenic conditions.

PKCδ - SPAK (STE20P-RELATED PROLINE ALANINE-RICH KINASE) INTERACTION IN THE REGULATION OF NATIVE HUMAN NKCC1

Laura Smith and Carole M. Liedtke. *Departments of Pediatrics and Physiology & Biophysics, Case Western Reserve University, Cleveland, Ohio 44106-4948 cx17@case.edu*

Activation of native NKCC1 by hyperosmotic stress involves a regulatory proteome which includes PKCδ interacting directly with the actin cytoskeleton and with SPAK kinase. NKCC1 activation requires phosphorylation, yet PKCδ does not directly phosphorylate NKCC1. The goal of this study is to determine whether SPAK is part of a kinase cascade linking PKCδ to phosphorylation of native human epithelial NKCC1. NKCC1 activity was measured as bumetanide-sensitive basolateral to apical 86-Rb flux in Calu-3 cells. SPAK was downregulated using siRNA delivered into Calu-3 cells using an Amaxa nucleofector. siSPAK reduced SPAK protein expression by 73.5% without altering expression of NKCC1, PKCδ, and actin and prevented activation of NKCC1 by hyperosmotic stress. Substitution of a non-targeting siCONTROL RNA did not affect expression of SPAK, PKCδ, NKCC1, and actin or activation of NKCC1. Using recombinant proteins, we demonstrate direct binding of PKCδ to SPAK and PKCδ-mediated activation of SPAK. In intact cells, hyperosmotic stress increased native phosphorylated PKCδ (pPKCδ) 2.4-fold, indicating activation of PKCδ, the amount of PKCδ and pPKCδ coimmunoprecipitating with SPAK 2.9-fold and 3.6-fold, respectively, and the amount of SPAK coimmunoprecipitating with PKCδ by 1.9-fold. The results indicate that PKCδ mediates the stress signal to SPAK. Supported by a grant from the NIH, RO1-HL-58598.

FoxO1 INDUCES APOPTOSIS IN SKELETAL MYOTUBES

Sierra Smith[1], Esser, K.A.[2], Unterman, T.G.[3], McLoughlin, T.J.[1]
[1]Dept. of Kinesiology, Univ. of Toledo, Toledo, OH, 43606 thomas.mcloughlin@utoledo.edu; [2]Dept. of Physiology, Univ. of Kentucky, Lexington, KY, 40506; [3]Medical Research Unit, Jesse Brown VA Medical Center, Chicago, IL, 60612. Corresponding Author: sierra.smith2@utoledo.edu

We recently demonstrated that the transcription factor FoxO1 promotes atrophy of skeletal myotubes in vitro, which was independent of DNA binding. The purpose of this study was to examine mechanisms of FoxO1-mediated muscle atrophy via gene expression analysis for genes associated with protein ubiquination (MAFbx, Murf-1), apoptosis (Bim) and autophagy (BNip3). FoxO1-estrogen receptor fusion proteins ($FoxO1^{AAA}$ and $FoxO1^{215}$ [DNA-binding deficient]) that are activated by treatment with 4-hydroxytamoxifen (4-OHT) were stably transfected in C2C12 skeletal myoblasts using the pBABE retroviral system and grown into 4-day-old skeletal myotubes. Non-transfected C2C12 cells served as controls. After 24 hour treatment with vehicle or 4-OHT, total RNA was isolated and gene expression performed using qPCR. Activation of $FoxO1^{AAA}$ resulted in a significant increase in MAFbx (~27 fold), and Bim (~3.5 fold) gene expression, with no significant increase in Murf-1 or BNip3 gene expression. Whereas, activation of the $FoxO1^{215}$ resulted in a significant increase in Murf-1 (~2.2 fold), and BNip3 (~2.2 fold) gene expression, with no significant increase in MAFbx or Bim gene expression. No change in gene expression was observed in the control cells. These findings demonstrate that muscle atrophy induced via FoxO1 activation is associated with the induction of genes responsible for regulating ubiquination, apoptosis and autophagy, via DNA binding dependent and independent mechanisms

UNDERSTANDING THE ROLE OF CONSTITUTIVE TRPC3 CHANNELS IN ATHEROGENESIS: AN INFLAMMATORY TR(i)P

Jean-Yves Tano, Kathryn Smedlund and Guillermo Vazquez
Department of Physiology and Pharmacology, University of Toledo College of Medicine Corresponding author: Jean-Yves.Tano@utoledo.edu

Atherosclerosis, a chronic inflammatory disease of the vascular endothelium, is the leading cause of death by cardiovascular events. Characterizing the molecular/cellular components involved in atherosclerotic lesion formation and progression is vital to identify new targets that can be used in designing alternative therapeutic strategies for the disease. Canonical Transient Receptor Potential (TRPC) channels are now emerging as critical players in cardiovascular disease, thus becoming attractive candidates for molecular targeting. We have recently identified TRPC3 as a novel player in the inflammatory response that occurs early in atherogenesis. For instance, in human coronary artery endothelial cells (HCAECs) maneuvers that prevent TRPC3-mediated Ca^{2+} entry into the cell or knock-down of native TRPC3 protein, markedly reduce expression of VCAM-1 and monocyte adhesion in response to pro-atherogenic stimuli (Smedlund & Vazquez, Arterioscler. Thromb. Vasc. Biol. in press). Notably, the dependency on Ca^{2+} influx or TRPC3 expression (see Abstract by Smedlund and Vazquez, this series) was manifest regardless of the ability of the stimulus to promote Ca^{2+} entry. In most cell types TRPC3 forms channels endowed with significant non-regulated, constitutive activity. HCAECs are not an exception as native TRPC3 forms Ca^{2+}-permeable channels that contribute not only to receptor-regulated but also to constitutive cation influx. Within this context, our findings suggest that non-regulated channel activity may have an impact in atherogenesis. Understanding the role of constitutive TRPC3 activity on downstream Ca^{2+}-dependent events that contribute to this or other pathophysiological processes in the endothelium, demands elucidation of the regulatory mechanisms underlying non-regulated channel function. Although some efforts have been made to understand the structural requirements associated to constitutive TRPC3 activity, information on the underlying signaling remains scarce. In the present studies we used human embryonic kidney cells (HEK293) stably expressing the human version of TRPC3, a well characterized TRPC3 overexpression system, to evaluate TRPC3-mediated constitutive cation influx by means of Fura-2 based real-time fluorescence. Ba^{2+} was used as a surrogate for Ca^{2+} to avoid complications due to Ca^{2+} buffering systems, and pharmacological maneuvers were applied to evaluate signaling pathways that might contribute to support TRPC3 constitutive activity. Our findings are discussed within the context of the potential impact of constitutive channel function on signaling events that contribute to atherogenesis.

Oral 12:00 Noon

MULTIPLE QUANTITATIVE TRAIT LOCI ON RAT CHROMOSOME 9 IN WHICH SPONTANEOUSLY HYPERTENSIVE RAT ALLELES CONFER A REDUCTION IN BLOOD PRESSURE OF A SALT-SENSITIVE MODEL

Edward J. Toland, Shane Yerga-Woolwine, Phyllis Farms, and Bina Joe. *Department of Physiology and Pharmacology, University of Toledo College of Medicine, 3035 Arlington Avenue, Toledo, Ohio 43614 Corresponding author: bina.joe@utoledo.edu*

A Blood Pressure (BP) quantitative trait locus (QTL) was previously located within 117kb on rat chromosome 9 (RNO9) using the hypertensive Dahl Salt-sensitive (S) rat and the normotensive Dahl Salt-resistant (R) rat. An independent study between two hypertensive rat strains, the S rat and the Spontaneously Hypertensive Rat (SHR) also detected a QTL encompassing this 117kb region. S alleles in both of these studies were associated with increased BP. To map the SHR alleles which decrease BP of the S rat, a panel of eight congenic strains introgressing SHR alleles onto the S genetic background were constructed and characterized. S.SHR(9)x3B, x3A and x2B, all of which span a portion or all of the 1-LOD interval identified by linkage analysis, did not significantly alter BP. However, S.SHR(9), S.SHR(9)x4A, x7A , x8A and x10A the introgressed segments of which extend distal to the 1-LOD interval, significantly reduced BP compared to S rats. The shortest deduced region of overlap between S.SHR(9)x4A and S.SHR(9)x8A was prioritized as BP QTL1, which also has an observed urinary protein excretion (UPE) effect. However, the introgressed segment of S.SHR(9)x10A, which was outside of this prioritized region, represented a second BP QTL with no UPE effect. Only one of these BP QTLs, BP QTL1 overlaps with the 117 kb QTL region identified using S and R rats. In summary, the data suggests that there are multiple RNO9 alleles of the SHR that lower BP of the S rat with disparate effects on urinary protein excretion.

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IDENTIFICATION OF OBESITY AND ADIPOSE DISTRIBUTION QTL IN A RAT MODEL OF OBESITY.

[1]Marlene Welch, [1]Sarah DeRaedt, [1]Andrea Kalinoski, [2]George Cicila, and [1]David Allison

Departments of [1]Surgery, [2]Physiology and Pharmocology, University of Toledo College of Medicine, 3035 Arlington Avenue, Toledo, Ohio 43614 Corresponding author: Marlene.Welch@utoledo.edu

INTRODUCTION: Obesity affects nearly one-third of adults in the United States. Obese adults are at increased risk for developing health problems including coronary artery disease, diabetes, hyperlipidemia, hypertension, cerebrovascular disease and cancer. Although several Mendelian obesity genes have been identified, these are rare causes of human obesity. Common obesity is multifactorial and the pathophysiology involves a complex interplay of genes and environment. Twin and adoptee studies suggest that up to 70% of obesity is genetic, however the genes responsible for polygenic obesity have not been identified. We have developed an animal model to dissect the complex genetic nature of obesity by identifying chromosomal regions (quantitative trait loci, QTL) linked to obesity.
METHODS: Two rat strains, non-obese Copenhagen (COP) and obese Buffalo (BUF) demonstrate a 5-fold difference in fat weight. The COP and BUF rats were bred for two generations in order to create a diverse population with a wide range of obese phenotypes. The animals were phenotyped according to the weight of the subcutaneous fat, visceral fat, retroperitoneal fat and total fat. To identify chromosomal regions that corresponded to the highest 20% (obese) rats and the lowest 20% (non-obese) rats, a genome scan was performed using 176 microsatellite markers.
RESULTS: We have identified four chromosomal regions that are linked with subcutaneous fat, visceral fat, total fat and adiposity index (total fat weight / body weight). Subcutaneous fat is linked to a 20cM region in the lower *q* arm of chromosome 1 between microsatellite markers D1rat452 and D1rat90. Visceral fat, total fat and adiposity index are linked to a 40cM region of rat chromosome 3 between microsatellite markers D3rat117 and D3rat227.
CONCLUSION: We have identified several chromosomal regions (significant QTLs) that are linked with the obese phenotype in our animal model. Many candidate obesity genes are located within these chromosomal regions, most notably the gene for insulin on chromosome 1 and several genes that regulate lipid metabolism on chromosome 3. Further studies will include creation of congenic animals and the use of microarray technology to examine the expression profiles of the various adipose tissue depots.

EPITHELIAL CELL SPECIFIC CAPSAICIN EFFECT ON Cl^- SECRETION AND NKCC1

Jeremy M. Jacobs[2], Mary Fedor-Chaiken[3], Lisa Merk[3], Eric Delpire[4], Jeffrey B. Matthews[5] and Roger T. Worrell[1]

[1]Dept. of Molecular & Cellular Physiology and Systems Biology & Physiology Graduate Program, [2]SURF Program, [3]Dept. Surgery, University of Cincinnati
[4]Dept. Anesthesiology and Molecular Physiology & Biophysics Vanderbilt University
[5]Dept. Surgery, University of Chicago. Corresponding: worrelrt@ucmail.uc.edu

Capsaicin concentrations well within the physiologic effective dose can be achieved in gut thus exposing the mucosa to this potential signaling molecule. Capsaicin effects on gut thus far have been attributed to a neuronal action. Since capsaicin treatment results in increased $[Ca^{2+}]_i$ via action on TRPV1 receptors in neurons, this study sought to determine if capsaicin has a direct effect on epithelial cells. In colonic T84 cells, Ca^{++}-agonist pretreatment results in attenuation of subsequent cAMP-stimulated Cl^- secretion. We have previously shown that this may, in-part, be explained by Ca^{++}-induced internalization of NKCC1. In T84, 5 min pre-application of 2μM capsaicin resulted in an attenuated response to forskolin-induced current similar to that obtained with pre-application of 100 μM CCh. Capsaicin IC_{50} was ~16 μM which is roughly equivalent to a mild pepper. In MDCK cells transfected with EGFP-NKCC1, Capsaicin (15-80 μM) induced visible internalization of EGFP-NKCC1 within 5 min. Apical capsaicin induced NKCC1 internalization, whereas basolateral capsaicin was ineffective. Capsaicin-induced NKCC1 internalization was dependent on the presence of extracellular Ca^{++}. These data support direct epithelial cell capsaicin effects on gut Cl^- secretion. Thus, capsaicin may be useful to limit Cl^- secretion and possibly promote HCO_3^- secretion via internalization of NKCC1. The clinical significance of this study is supported by reports of TRPV1 up-regulation in Inflammatory Bowl Disease and colitis. Furthermore, $TRPV1^{-/-}$ mice have altered susceptibility to various forms of induced-colitis. Particular relevance to human gut function is accentuated by many persons consuming capsaicin at levels well within the range used in this study.

ECCENTRIC CONTRACTION IN *mdx* MICE TRABECULAE

Ying Xu, Paul M.L. Janssen

Department of Physiology and Cell Biology, The Ohio State University, Columbus, OH. Correspondence to janssen.10@osu.edu

Duchene muscular dystrophy (DMD) is an inherited and progressive disease of striated muscle deterioration. It is caused mainly by mutations with no or little production of functional dystrophin protein, which connects the cytoskeleton of a muscle fiber to extracellular matrix through the cell membrane. Compared with studies on skeletal muscle, which most of the molecular basis of DMD has come from, little attention has been directed to cardiac muscle. Since patients with DMD are living longer into adulthood due to improvements in therapeutic treatments, it becomes increasingly important to have cardiomyopathy anticipated, detected and treated. It has been shown previously that dystrophin-deficient myocytes have reduced compliance and increased susceptibility to stretch-mediated calcium overload. To detect the role of mechanical stress in dysprophin-deficient myocardium under more physiological conditions, we used a computer programmed protocol to trigger eccentric contraction at 4 Hz in age matched *mdx* and wild type mice right ventricular trabeculae. These ultra-thin muscles possess all major cardiac cell types and their contractile behavior very closely mimics that of the whole heart. In the first group of experiment, 10 eccentric contractions were performed in trabeculae from 10 week old *mdx* and wild type mice. In the second group of experiment, 100 eccentric contractions were conducted in trabeculae from 20 week old *mdx* mice. The peak isometric active developed tension (F_{dev}, in mN/mm^2), diastolic tension (F_{dia}), and the force run-down were measured throughout the whole protocol. The baseline F_{dev} for *mdx* is 3.02 ± 0.23 lower than wild type mice. The force run-down data were presented by normalizing with baseline developed force respectively. The results of 10 week old mice do not show differences in force run-down data, except at the end of protocol *mdx* is slightly lower than wild type (not statistically significant, n=8). The results of 20 weeks old mice show F_{dev} went down quickly in *mdx* mice (n=2). Our results indicate that shorter period eccentric contractions show minor impact on myocardium of younger *mdx* mice, while longer and severe mechanical stress is more damaging in older *mdx* mice.

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Matthew Dilworth
ADInstruments
2205 Executive Circle
Colorado Springs, CO 80906-4137
mdilworth@adinstruments.com
415 378-0986

Scott Evans
Qiagen
19300 Germantown Road
Germantown, MD 20874
scott.evans@quigen.com
734-233-1670,

Richard Goodman
Hatteras instruments, Inc.
105 Southbank Drive
Cary, NC 27518
rg@hatterasinstruments.com
919-816-9905

Gary Kurk
Data Science
119 14th Street, NW
St. Paul, MN 55112
gkurc@datasci.com
651-414-5423,

Jim Nowak
Promega
2800 Woods Hollow
Madison, WI 53711
jim.nowak@promega.com
800-356-9526-1429

Jason Arbinger, Jason
Denville Science
411 E. Broadway Street
Maumee, Ohio 43537
Jason@denvillescientific.com
419-450-7760