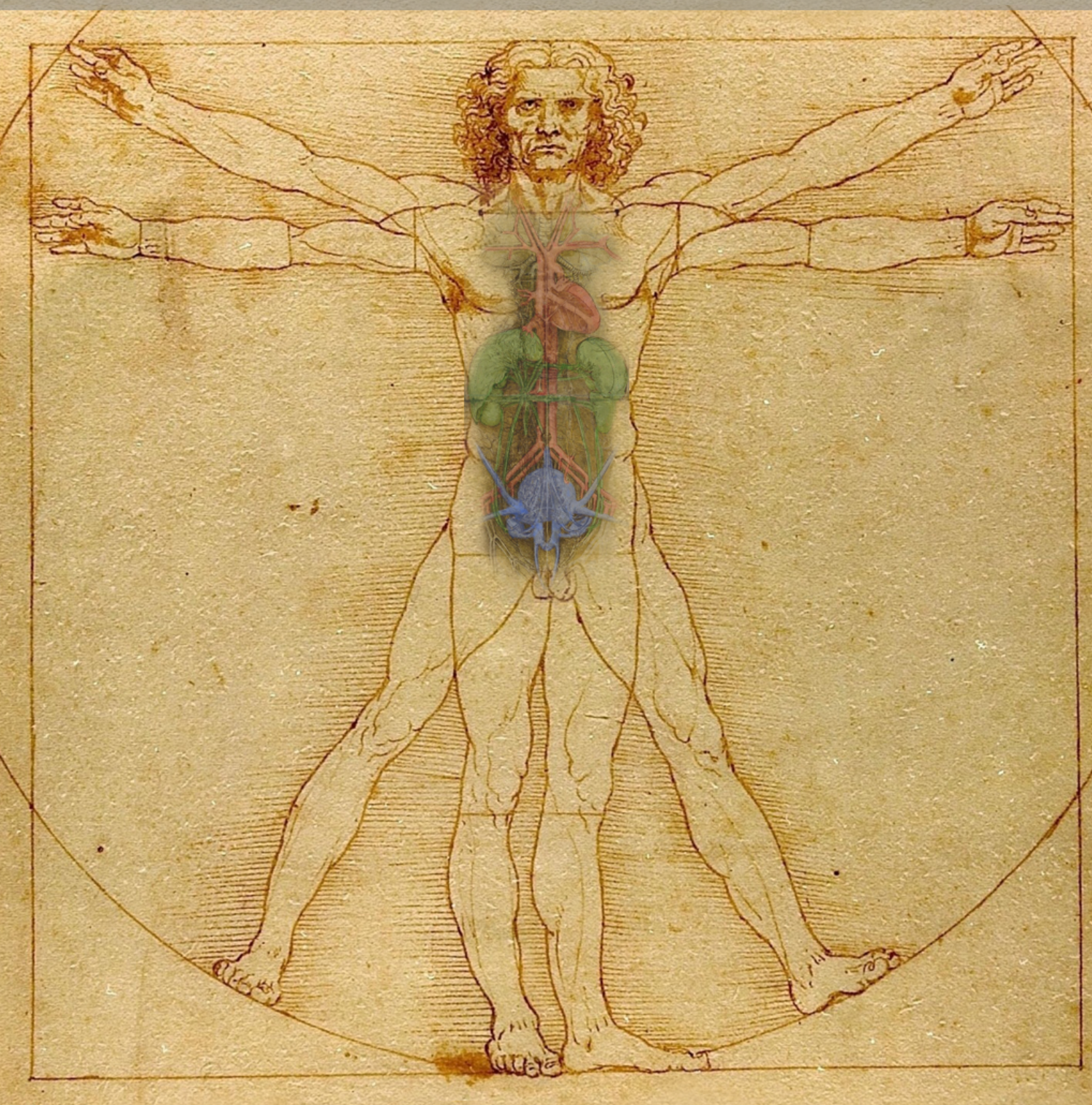
33rd Annual

FRONTIERS IN PHYSIOLOGY



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Frontiers in Physiology Schedule

8:00 – 8:45 **Registration & Breakfast**
Croft Chapter House

8:50 – 9:00 **Opening Remarks** – J. Menchella, M. Kwan & Dr. S. Matthews
Theatre – UC140

9:00 – 9:45 **Cardiovascular Platform Oral Presentations**
Theatre – UC140

F. Malik	Treating the detrimental effects of TNF-alpha in heart failure with a CFTR-specific small-molecule corrector compound
H. Kim	The role of protein kinase C delta (PKCΔ) in modulating cellular and molecular mechanism of ischemia-reperfusion-induced lung injury
P. Wood	Scaffold protein CARD11 modulates receptor mediated apoptosis in the myocardium following myocardial infarction

9:45 – 10:00 **Break**

10:00 – 10:45 **Reproduction & Development Platform Oral Presentations**
Theatre – UC140

S. Baello	Transforming growth factor-β1 is a potent activator of drug transport in the fetal blood-brain barrier (BBB)
T. Nguyen	Characterizing the gene expression pattern of myometrial MMPs and TIMPs throughout pregnancy, labour and post-partum
J. Jiang	Modulation of DNA methylation and phenotypic switching in Smooth Muscle Cells by damaged matrix

10:45 – 11:00 **Break**

11:00 – 12:00 **KEYNOTE LECTURE**
Theatre – UC140

"Systems Medicine and Emerging Technologies: Catalyzing Proactive P4 Medicine"

Dr. Leroy Hood, MD, PhD
President, Institute for Systems Biology

Frontiers in Physiology Schedule

12:00 – 12:30 **Departmental Photo**
MSB Steps, 1 King's College Circle

12:30 – 1:00 **Poster Set-Up & Lunch**
UC East Hall, Croft Chapter House

1:00 – 2:15 **Poster Viewing & Judging**
UC East Hall

2:15 – 3:00 **Endocrine and Diabetes Research Group Oral Presentations**
Theatre – UC140

T. Odisho	ATF6β depletion increases susceptibility of pancreatic β-cells to endoplasmic reticulum stress-induced apoptosis
F. Dang	miR-142-3p directly regulates autophagy-dependent gene ATG16L1 in Crohn's disease
J. Oosterman	Effects of timing of saturated fat and liquid sugar intake on obesity in rats and circadian rhythms in hypothalamic cells

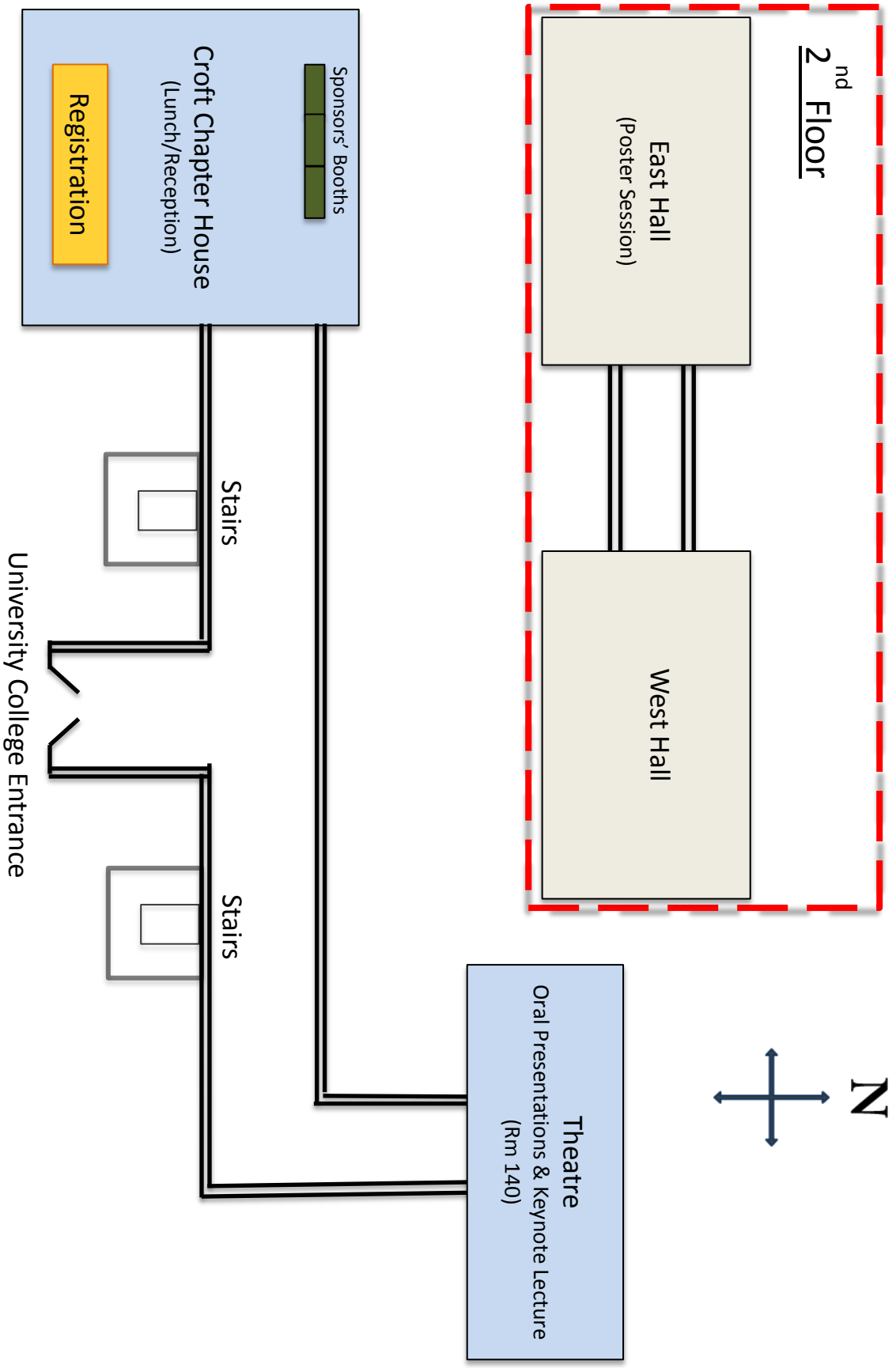
3:00 – 3:15 **Break**

3:15 – 4:00 **Brain and Integrated Neurophysiology Platform Oral Presentations**
Theatre – UC140

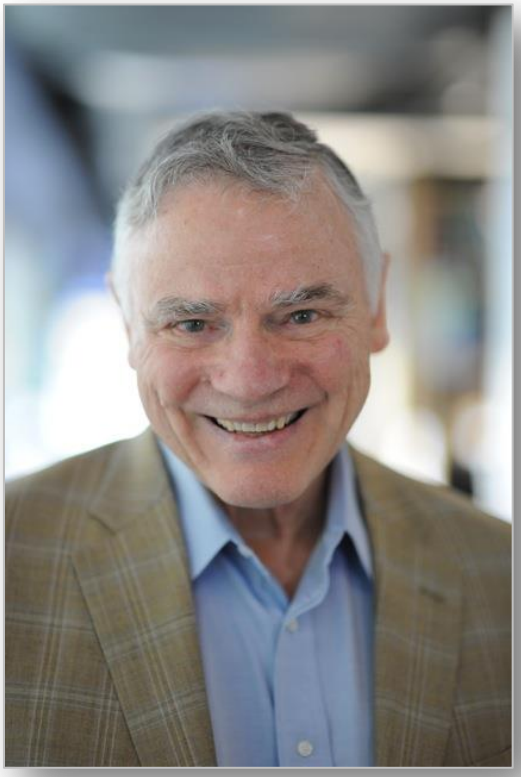
G. Rozanski	The dorsal root ganglion sandwich synapse: novel transglial signaling between neuronal somata
K. Grace	Reconciliation of the Reciprocal Interaction and Flip-flop Models of Rapid Eye Movement sleep generation
A. Mosa	GABAergic trophic signaling in hippocampal neurogenesis: examining the in-vitro effects of selective $GABA_AR$ agonists

4:00 – 6:00 **Awards Ceremony & Reception**
Croft Chapter House

Frontiers in Physiology Floor Plan



Keynote Speaker



Dr. Leroy Hood, MD PhD

President, Institute for Systems Biology

One of the world's leading scientists, Dr. Hood began his scientific career at the California Institute of Technology, obtaining a PhD in Biochemistry in 1968. At Caltech, Dr. Hood and his colleagues invented the DNA and protein sequencer and synthesizer, four machines that revolutionized the field of molecular biology and genomics. In particular, enabling rapid automation of DNA sequencing paved the way for the mapping of the human genome, of which Dr. Hood has been an integral part. In 2000, Dr. Hood founded the Institute of Systems Biology, where genomics, transcriptomics, proteomics, and metabolomics are integrated to further current knowledge of systems biology. Dr. Hood is an expert in adaptive immunity, genomics, biotechnology, systems biology, and personalized medicine. He is a member of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine, and possesses the great honour of being one of only 15 scientists in the world who are members of all three institutions. He has published over 700 papers, and is the recipient of several prestigious awards, including the Albert Lasker Award, the Lemelson-MIT Prize, the Kyoto Prize, the Heinz Award, the NAE 2011 Fritz J. and Delores H. Russ Prize, and most recently, the National Medal of Science.

MESSAGE FROM THE

FRONTIERS IN PHYSIOLOGY CO-CHAIRS

On behalf of the Graduate Association of Students in Physiology (GASP), we are happy to welcome you to the 33rd Annual Frontiers in Physiology (FIP), which showcases the cutting-edge research being conducted across all four platforms within the Department of Physiology and facilitates exchange of scientific ideas amongst students and faculty members within the University of Toronto and its affiliated teaching hospitals and research institutions.

Each year, the Department of Physiology invites a distinguished speaker to present a keynote lecture at FIP. This year, we welcome **Dr. Leroy Hood** to the University of Toronto, current President of the Institute of Systems Biology. Dr. Hood's work has revolutionized molecular biotechnology and genomics, and we are honoured to have him deliver a lecture entitled "Systems Medicine and Emerging Technologies: Catalyzing Proactive P4 Medicine".

Today's events would not be possible without the contributions of several individuals. Firstly, we would like to acknowledge the members of the FIP Planning Committee and GASP, whose assistance and efforts have ensured the success of FIP. Additionally, we are truly grateful for the continuing support of the Department of Physiology, particularly our chair, **Dr. Stephen Matthews**; the Graduate Coordinators, **Drs. Denise Belsham** and **Martin Wojtowicz**; and **Jenny Katsoulakos** and the rest of the departmental administrative staff. We would also like to thank all the trainees participating in FIP today, as well as the faculty members and postdoctoral fellows who have graciously volunteered their time to assist in judging their presentations. Finally, many thanks must be extended to our institutional and commercial sponsors for their financial support: the Department of Physiology Research Platforms, the Fraser Mustard Institute of Human Development, the Faculty of Medicine, and the Graduate Students' Union of the University of Toronto, UTPoster.com, Cedarlane, Bio-Rad, Eppendorf, and Life Technologies/Invitrogen.

We are delighted to have you with us today.



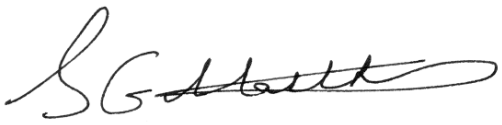
Jonathan Menchella and Melissa Kwan
Vice-Presidents, Graduate Association of Students in Physiology
Co-Chairs, Frontiers in Physiology

MESSAGE FROM THE CHAIR OF THE DEPARTMENT OF PHYSIOLOGY

On behalf of the Department of Physiology, it is a very great pleasure to welcome you to the annual **"Frontiers in Physiology Research Day"**. The annual FIP Symposium is our primary research day in the Department and it is a high point of the academic year. It has been organized by our graduate students for the past 33 years. Today will highlight the enthusiasm, originality and creativity of our trainees who represent the future of our discipline and of biomedical research in Canada and around the world. We are extremely proud of their research accomplishments and dedication to science, and look forward to an outstanding day of enlightening presentations and discussions.

Special thanks and recognition is due today to **Ms. Melissa Kwan** and **Mr. Jonathan Menchella,** FIP co-Chairs and Vice-Presidents of the Graduate Association of Students in Physiology (GASP). Melissa and Jonathan's team have done an outstanding job and have generated an excellent program. Please join me in also thanking **Mr. Lemieux Luu**, President of GASP.

We hope you enjoy the day which is dedicated to the future of physiological research and brought to you by our Graduate Students!



Stephen G. Matthews, PhD
Ernest B. and Leonard B. Smith Professor and
Chair, Department of Physiology
Professor, Physiology, Obstetrics & Gynaecology and Medicine
Faculty of Medicine
University of Toronto



Cardiovascular Platform

Cardio: Oral Presentations

TREATING THE DETRIMENTAL EFFECTS OF TNF-ALPHA IN HEART FAILURE WITH A CFTR-SPECIFIC SMALL-MOLECULE CORRECTOR COMPOUND

F. Malik[1], A. Meissner[1], M. Sauvé[1], J. Kroetsch[1], C.E. Bear[1,2], St-S. Bolz[1]
Department of Physiology[1], University of Toronto, Toronto, Canada; Molecular Structure and Function[2], The Hospital for Sick Children, Toronto, Canada

Resistance artery tone is tightly regulated by sphingosine-1-phospate (S1P). Our lab showed the Cystic Fibrosis Conductance Regulator (CFTR) chloride channel uptakes S1P for degradation. Tumor-necrosis alpha (TNF-alpha) reduces S1P-uptake through down-regulation of CFTR expression in heart failure, leading to an increase in microvascular tone. The present study proposes that CFTR-specific small-molecule corrector compounds can reverse the effects, thereby providing therapeutic potential for heart failure. Treatment of BHK cells (stably expressing human WT-CFTR) with TNF-alpha (10 ng/mL) showed a 75% reduction in channel activity, assessed by the conventional iodide efflux assay. C18, a corrector compound, reversed the down-regulation of channel activity ($p<0.05$). Moreover, in cultured murine vascular smooth muscle cells (VSMCs) C18 treatment reversed the TNF-alpha-mediated reduction of endogenous CFTR protein expression, correlating to similar effects on S1P-uptake. Congestive heart failure (CHF) mice, produced through ligation of the left anterior descending (LAD) coronary artery, were then used to assess C18's ability to reverse the increased myogenic tone seen in CHF. Assessment of myogenic tone was done in posterior cerebral arteries (PCAs) isolated and cannulated from the SHAM and CHF mice. We found that i.p. injection with C18 for two-days prior to sacrifice lead to a reversal in the enhanced the myogenic tone of CHF mice ($p<0.05$), and correlating nicely with increased cerebral blood flow (CBF). Finally, CFTR-specificity of C18's effect on myogenic tone was assessed in PCAs isolated from delta-F508 mice, the most common disease causing CFTR mutation. The mutants had almost 50% higher myogenic tone ($p<0.05$) relative to WT mice, and in-bath C18 treatment it to near WT levels ($p<0.05$). Given that C18 reverses the TNF-mediated down-regulation of CFTR function and expression, the therapeutic potential was shown with excitement in CHF mice and as well as the delta-F508 mice. Thus our study suggests that compounds being developed for the Cystic Fibrosis diseases have potential for the heart failure diseases as well.

THE ROLE OF PROTEIN KINASE C DELTA (PKCΔ) IN MODULATING CELLULAR AND MOLECULAR MECHANISM OF ISCHEMIA-REPERFUSION-INDUCED LUNG INJURY

H. Kim[1,2,3], J. Zhao[1,2], D. Lee[1,2,4], Y. Wang[1], L. Turrell[1,8], Q. Zhang[1], S. Keshavjee[1,2,6,7], M. Liu[1,2,3,4,5]
Latner Thoracic Surgery Research Laboratories[1], Toronto General Research Institute[2], Department of Physiology[3], Institute of Medical Science[4], Department of Surgery[5], Division of Thoracic Surgery[5], and Institute of Biomaterials and Biomedical Engineering[7], University of Toronto; and Department of Biochemistry[8], Oxford University.

Ischemia-reperfusion (IR) induced lung injury is one of the major causes of patients' morbidity and mortality after lung transplantation. PKCδ, a novel PKC subtype, mediates intercellular signaling pathways related to inflammatory response in many organs. We recently reported that PKC activation alone can induce cytoskeletal reorganizations, cell death and cytokine production in human lung epithelial cells. Moreover, selective PKCδ inhibition is known to ameliorate IR-induced injury in the brain and heart. Thus, we hypothesize that PKCδ activation is an important signaling event in IR-induced lung injury. We examined PKCδ phosphorylation and activity in lung tissues collected from clinical lung transplantation. A cell culture model with human normal bronchial epithelial (BEAS-2B) cells was used to determine the potential role of PKCδ in hypothermic IR-induced inflammatory response and cell death. A rat pulmonary in situ IR model was used to test the efficacy of PKCδ inhibition in improving the lung function. The PKCδ phosphorylation and activity was higher in human lung tissue collected after cold ischemic periods (CIT) and significantly decreased during reperfusion. In cell culture, PKCδ was activated after both 6 and 18 h CIT. Hypothermic IR-induced dramatic morphological changes, inflammatory cytokine production and cell death were effectively reduced by PKCδ siRNA or by a specific peptide inhibitor (δV1-1) in human lung epithelial cells. Lastly, administration of δV1-1 effectively prevented deterioration of lung functions and cell death during reperfusion in the rat pulmonary in situ IR model. Subsequent biochemical analysis suggested that necrotic cell death was significantly inhibited. Hypothermic preservation of donor lung induced PKCδ activation is an important signaling event that may contribute to IR-

induced lung injury by enhancing inflammation and necrotic cell death. Thus, selective inhibition of PKCδ may present an effective therapy to prevent IR-induced lung injury in transplantation.

SCAFFOLD PROTEIN CARD11 MODULATES RECEPTOR MEDIATED APOPTOSIS IN THE MYOCARDIUM FOLLOWING MYOCARDIAL INFARCTION

P. Wood[1,3], M. Moon[2,3], L. Zhang[3], F. Dawood[3], M. Nishi[3], K. Naito[3], P. Liu[1,2,3].
Department of Physiology[1], Institute of Medical Science[2], Faculty of Medicine, University of Toronto, 1 King's College Circle, Toronto, Ontario, Canada. University Health Network[3], Toronto, Ontario, Canada.

CARD scaffold superfamily members are important modulators of inflammatory and apoptotic signalling. CARD11, a membrane associated scaffold, is essential for the production of cytokines in the immune cellular response but is present in heart and lung tissue, where its effects are unknown, and is described to associate with apoptotic players such as BCL10 and Caspase-8. Due to the complex nature of cardiac response to myocardial infarction (MI), we hypothesize that CARD11 can act as a modulator of receptor mediated apoptotic pathways following cardiac tissue injury. Experimental MI was created in CARD11-deficient mice and wild-type littermate controls by left coronary artery ligation. Survival over 28 days was significantly improved in $CARD11^{-/-}$ (95%) over littermates (47%) and reduced infarct size. $CARD11^{-/-}$ mice exhibited lower rates of rupture prior to day 7 post-MI. TUNEL staining identified a lower percentage of apoptotic cells in the border zone of $CARD11^{-/-}$ hearts post-MI and western blot analysis displayed decreased levels of pro-apoptotic proteins caspase-3, -8, -9, Apaf1, cytochrome-C and Bid. CARD11 expression during this period is significantly increased in the myocardium and is paralleled by general stress induction by H2O2 (25μM) and death receptor activation by Fas agonist Jo2 (1μg/ml) and TNF-alpha (50ng/ml) in isolated neonatal cardiomyocytes. Lack of CARD11 in vitro demonstrated decreased number of apoptotic cells following H2O2, Jo2 and TNF-alpha treatments compared to wild-type, analyzed by Annexin-V – propidium iodide double stained flow cytometry. Pre-treatment with caspase inhibitor zVAD-fmk (25μM) blocked apoptosis in $CARD11^{+/+}$ and replacement of CARD11 through plasmid transfection restored the apoptotic phenotype in $CARD11^{-/-}$ cells. From these results, the recruitment of CARD11 to death receptor signalling complexes propagates apoptotic signalling through caspase activation in the infarct and border zone and its removal can provide protection against cardiomyocyte death and cardiac rupture.

Cardio: Posters

C1. THE TUMOR NECROSIS FACTOR ALPHA/SPHINGOSINE-1-PHOSPHATE SIGNALLING AXIS MEDIATES AUGMENTATION OF MICROVASCULAR TONE IN A MOUSE MODEL OF TYPE 2 DIABETES MELLITUS

M. Sauvé[1] and St.S. Bolz[1]
Department of Physiology[1], Faculty of Medicine, University of Toronto, Ontario, Canada

Diabetes is a primary risk factor for cardiovascular disease, and is associated with increased mortality due to the complications of widespread end-organ damage (e.g. renal dysfunction, heart failure, cerebrovascular ischemia). The pre-capillary resistance arteries, key regulators of tissue perfusion, are targets of metabolic dysregulation. Compromised endothelial signaling has been implicated as a cause of microvascular dysfunction, however, this approach has not yet yielded effective therapies. This may result from the role for microvascular smooth muscle cells (MVSMCs) as *direct* targets of diabetes-induced pathological processes. MVSMCs convert transmural pressure into biochemical signals to regulate contractility, termed the *myogenic response*. Sphingosine-1-phosphate (S1P) is a central regulator of the myogenic response through the pressure-induced activation of the S1P producing enzyme sphingosine kinase 1 (SphK1) and the $S1P_2$ receptor. Recently, TNFα has been shown to augment the myogenic response of resistance arteries in diseases that share an inflammatory component (e.g. heart failure). As diabetes is associated with increased TNFα, *we propose that activation of the TNFα/S1P signaling axis contributes to microvascular dysfunction in diabetes*. Mice were treated with high fat diet (HFD) + streptozotocin to induce type 2 diabetes (T2D). T2D mice had elevated HbA_{1C} levels compared to HFD controls. T2D mice show augmented myogenic tone in isolated mesenteric, posterior cerebral, and olfactory resistance arteries, while resistance arteries isolated from HFD mice did not display altered myogenic tone even after one year on HFD. T2D did not alter artery responses to phenylephrine. These data suggest that hyperglycemia/diabetes, not obesity, specifically alters the myogenic response in MVSMC. TNFα was implicated, as acute *in vitro* treatment with the TNFα scavenger etanercept attenuates the augmented myogenic response in isolated resistance from T2D mice. Chronic TNFα inhibition by 1) *in vivo* etanercept treatment or 2) genetic deletion of TNFα also normalized the myogenic response in T2D. A critical role for S1P was implicated as elevated myogenic tone in T2D was normalized by the $S1P_2$ receptor antagonist JTE013 and in resistance arteries from diabetic $SphK1^{-/-}$ mice. Our data suggest that the TNFα/S1P signaling axis regulates diabetes-induced augmentation of the myogenic response and identifies potential therapeutic targets to mitigate microvascular dysfunction in diabetes.

C2. NANOFORMULATION STRATEGY FOR THERAPEUTIC AGENTS IN PREVENTION OF ACUTE LUNG INJURY

S. Pacheco[1, 3], S.Y. Fung[3], K. Chen[1, 3], M. Liu[1, 2, 3]
Department of Physiology[1], Institute of Medical Science[2], Faculty of Medicine, University of Toronto, Ontario, Canada; Latner Thoracic Surgery Research Laboratories[3], University Health Network, Ontario, Canada

Acute lung injury (ALI) is a major complication of lung transplantations and is fundamentally the result of inflammation and oxidative stress. It is known that Src protein tyrosine kinases (PTK's) play an important role in the onset of such acute inflammatory responses. The hydrophobic Src PTK inhibitor, PP2, shows promise in prevention of multiple types of ALI through anti-inflammatory effects; however, its solubility relies heavily on toxic solvent dimethyl sulfoxide (DMSO). Our lab has explored nanoscale drug formulation strategies to provide clinical means of delivery for drugs such as PP2 which replaces toxic solvents. We hypothesize that a novel strategy using nanoscale combinations of self-assembling peptides and amino acids serves as an effective delivery vehicle for multiple hydrophobic drugs including PP2. To explore the potential of this strategy, 480 PP2 nano-formulations were screened primarily on the basis of PP2 solubility; each formulation consisting of a different combination/ratio of 1 of 6 self-assembling peptides with 1 of 20 amino acids. Results showed 60 of the 480 combinations successfully dissolved PP2. Soluble nano-formulations were further characterized by Dynamic Light Scattering (DLS) in order to determine nanosize, and individual therapeutic efficacies were compared in a high-throughput screen using a thrombin-induced injury model in BEAS-2B cells. These results narrowed down the number of PP2 nano-formulations to 2 – both of which had high efficacy comparable to PP2 dissolved in DMSO and did not cause cell death by MTS. The biological effects of Src PTK inhibition by PP2 in these nano-formulations was further confirmed by other experiments in vitro. A rat model of ALI is currently being investigated to demonstrate the potential of these PP2 nano-formulations in preventing ALI in vivo. It is also noteworthy that our strategy has proved its versatility through solubility of the hydrophobic anti-cancer drugs Rottlerin and Curcumin.

C3. SODIUM-COUPLED NEUTRAL AMINO ACIDS MEDIATE ALVEOLAR FLUID FLUX

S. K. Rozowsky[1,2], W. M. Kuebler[1,2]
Department of Physiology[1], Faculty of Medicine, University of Toronto, 1 King's College Circle, Toronto, Ontario, Canada; Li Ka Shing Knowledge Institute[2], St. Michael's Hospital, 209 Victoria St., Toronto, Ontario, Canada

Alveolar fluid clearance (AFC) is a physiological process by which the lungs remove fluid from the alveolar space by active absorption that is driven by epithelial Na^+ uptake. In isolated lungs, inhibition of the apical epithelial sodium channel (ENaC) with amiloride blocks AFC, and can reverse transepithelial ion and fluid transport to induce alveolar fluid secretion (AFS). In vivo, however, the scenario is considered more complex due to the presumed contribution of epithelial Na^+-cotransporters to AFC. Here, we specifically considered the role of the Na^+-coupled neutral amino acid transporter (SNAT). We aimed to analyze i) whether SNAT functionally contributes to AFC in the intact lung, ii) whether stimulating SNAT may attenuate AFS, and iii) whether inhibition of SNAT may stimulate AFS. AFC was determined by a double indicator dilution technique in the isolated perfused rat lung. Alveolar fluid flux is calculated from the concentration change of a high and low molecular weight fluorescent marker in the alveolar and vascular space, respectively, based on a two-compartmental distribution model. Neither stimulation of SNAT with the SNAT substrate L-alanine (5 mM) nor SNAT inhibition by $HgCl_2$ (100 µM) altered AFC in isolated lungs under basal conditions. Yet, when L-alanine was given along with amiloride, it was able to rescue AFC (0.19 ± 0.10 ml/h; $p < 0.05$ vs. amiloride -0.11 ± 0.17ml/h) and this rescue was abolished by $HgCl_2$ ($p<0.05$ vs. L-alanine and amiloride). Our findings indicate that SNAT does not critically contribute to AFC under basal conditions, yet provides an important rescue mechanism in scenarios in which ENaC-mediated Na^+ uptake is impaired or blocked. Stimulation of SNAT may therefore present an attractive novel strategy for reversing pulmonary edema.

C4. EFFECT OF SLC6A14 IN CFTR MEDIATED FLUID TRANSPORT AND PROLIFERATION OF INTESTINAL EPITHELIUM

S. Ahmadi[1,2,3], C. Luk[2], T. Chung[2], R. Mangat[1,2], J. Rommens[3], C. Bear[1,2]
Department of Physiology[1], University of Toronto; Molecular Structure and Function[2], Molecular Genetics and Genome Biology[3], The Hospital for Sick Children, Ontario, Canada

Cystic Fibrosis (CF) is considered to be a single gene disorder, most commonly the result of the F508del mutation in the CFTR gene. The CFTR protein functions as an anion channel to drive fluid secretion across the apical membrane of epithelial cells. The F508del-CFTR mutation causes protein misfolding, leading to the failure of CFTR to be functionally expressed in epithelial membranes. The epithelial surface becomes dehydrated and prone to inflammation and infection. Interestingly, patients homozygous for F508del mutation show a considerable variation in the severity of their disease. Consistent with this observation, recent genome wide association studies indicate the role of modifier genes in influencing

the disease phenotype. A Single Nucleotide Polymorphism in the putative promoter region of SLC6A14 gene showed the highest significance ($p=1.28x10^{-12}$). SLC6A14 transports 2 Na^+, 1 Cl^- and neutral/cationic amino acids across the apical surface of epithelial cells. Being a broad spectrum amino acid transporter, we hypothesize that it may contribute to proliferation and restitution of cystic fibrosis affected epithelium. We also hypothesize that solute transport via SLC6A14 modifies CFTR mediated fluid transport. Initially, to test if SLC6A14 affects CFTR function, we used heterologous expression system of BHK cells over-expressing SLC6A14 and WT or F508del CFTR. Using a fluorophore based halide flux assay (6-Methoxy-*N*-(3-Sulfopropyl)Quinolinium), we found that over-expression of SLC6A14 enhances CFTR mediated chloride flux across the membrane of these cells (~1.6 fold change for F508del CFTR). Further, to test the effect of SLC6A14 on cell proliferation and fluid secretion, we developed a three dimensional cyst based assay, using CaCo2 cells. Our preliminary results indicate that pharmacological inhibition of SLC6A14 using α-methyl-tryptophan decreases the size and number of cysts, and hence the fluid secreting capacity. This indicates that SLC6A14 affects CFTR mediated fluid secretion and might affect the restitution of intestinal epithelium. (Studies supported by CFC, CIHR, UofT)

C5. TNFα IS A CENTRAL REGULATOR OF SKELETAL MUSCLE RESISTANCE ARTERY MYOGENIC RESPONSIVENESS IN HEALTH AND HEART FAILURE

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Myogenic responsiveness (MR) is the property of resistance artery smooth muscle cells to oppose stretch through vasoconstriction. MR is the primary mechanism regulating peripheral resistance; its augmentation in heart failure (HF) compromises tissue perfusion. In cerebral arteries from HF mice, enhanced MR and reduced cerebral blood flow are governed by an autocrine effect of TNFα. Therefore, we hypothesize that (i) TNFα is an integral part of the mechano-sensitive chain (i.e., HF augments a physiological mechanism) and ii) TNFα drives augmented peripheral resistance in HF by augmenting skeletal muscle resistance artery (sRA) tone. Sequestration of TNFα with etanercept (ETN) reduced MR, intracellular [Ca^{2+}], and MLC_{20} phosphorylation in sRAs from four species (mouse, hamster, dog, and human). ETN did not affect these parameters after boiling or in sRAs from $TNF\alpha^{-/-}$ mice. Exogenous TNFα and inhibition of the TNFα converting enzyme by TAPI-1 failed to affect MR in sRAs. These manipulations did not affect vasoreactivity to phenylephrine, norepinephrine, and acetylcholine. In fully established murine HF, ETN corrected the augmented MR in sRAs. sRAs from $TNF\alpha^{-/-}$ HF mice did not show increased MR. Our data suggest that TNFα is an essential component of the mechano-sensitive chain regulating MR in sRAs. This mechanism is conserved over four different species. The lack of a TAPI-1 or (exogenous) TNFα effects suggests a sensing mechanism independent of TNFα shedding. We propose that HF recruits this *physiological* mechanism to sustain the compensatory increase in peripheral resistance that stabilizes systemic blood pressure at the cost of tissue perfusion.

C6. TRANSCRIPTOMIC AND PROTEOMIC BIOINFORMATIC ANALYSIS OF HUMAN FETAL AND ADULT ATRIAL AND VENTRICULAR CHAMBERS

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Atria (A) and ventricles (V) differ functionally and structurally in order to regulate mechanical and electrical activities in a coordinate fashion. There is an emerging need for bioinformatic analysis to characterize the differences between A and V and discover novel chamber specific and enriched genes and proteins for cell sorting and understanding of cardiovascular physiology, expression changes between fetal and adult hearts were also examined. Microarray expression and mass spectrometry based proteomics for 20 week human fetal heart chambers done previously and online adult A and V microarrays, from patients underwent aortic valve replacement, were analyzed. Global gene expression reveals that the two A chambers in fetal and adult have a stronger correlation than the two V chambers, and vice versa. Gene and protein expression do not correlate very well, however, in-depth comparison reveals that significantly upregulated (more than two-fold) genes for V in microarray and proteome were enriched in mitochondrial and cytoskeletal proteins by gene ontology. These genes provide the structure and energy required for these energy intensive pumps. Upregulated genes in A were enriched in extracellular proteins from proteome and plasma membrane proteins with many involved in signal transduction from microarray. Several well-known markers for A were also found in the dataset including SLN, connexin40, Kv1.5, and HCN1. Known markers for V include connexin43, MYH7, IRX4, and myosin light chains. In addition, a list of genes enriched in right A and left V at fetal, adult, and both stages were obtained. In order to further prioritize the candidate genes for validation, mouse homologues, cardiomyopathy datasets, and antibody availability were used as ranking criteria for selection of chamber specificity. Protein expression of these genes will be confirmed via immunofluorescence and immunohistochemistry with mouse and human heart tissue for chamber specific therapeutic targeting and role in heart diseases.

C7. ABSENCE OF DJ-1 ATTENUATES MORBIDITY AND MORTALITY IN EXPERIMENTAL SEPSIS

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Severe sepsis remains a leading cause of mortality in critically ill patients. Multi-organ dysfunction syndrome is a hallmark of severe sepsis. Bioinformatic analyses conducted in our lab identified mitochondrial pathways related to the Parkinson disease (autosomal recessive, early onset) 7 [PARK-7] or DJ-1 gene as significantly altered in experimental sepsis model. Serum DJ-1 levels were significantly increased in septic patients compared with control ICU patients; increased levels correlated with worse organ function outcome and higher mortality. To investigate the role of DJ-1 in sepsis, we randomized male wild-type (WT) C57Bl/6J and DJ-1null ($DJ\text{-}1^{-/-}$, knockout) mice (11 - 16 weeks) to sham or cecal ligation and perforation (CLP) surgery, as a clinically relevant model of sepsis. Post-operatively animals received fluid resuscitation and pain management every 12 hrs. Mortality was determined at 7 days. Organ dysfunction was assessed (i) histologically, (ii) functionally and (iii) biochemically at 24 and 48 hrs. Bacterial clearance and phagocytosis were determined *in vivo* and in isolated primary macrophages. DJ-1 deficiency resulted in a marked decrease in CLP-induced mortality at both 48 hours and 7 days. Surprisingly, despite improved survival, DJ-1KO mice had increased ROS and pro-inflammatory response compared to WTs. This was accompanied by an enhanced anti-inflammatory response as evidenced by increased anti-oxidant response in DJ-1 KOs. DJ-1KOs did not develop the typical decrease in ejection fraction (EF), fractional shortening (FS) and maximum rate of left ventricular pressure (+dP/dt) associated with sepsis-induced myocardial depression. In addition, bacterial counts in spleen were also markedly decreased in DJ-1KO compared with WT mice. This was in keeping with increased phagocytosis seen in DJ-1KO bone marrow derived (BMM) and peritoneal macrophages. Taken together our data suggests that (1) early efficient source control in the form of bacterial clearance and (2) heightened mechanisms of protection from injury may underscore survival from sepsis.

C8. ASSESSING THE ROLE OF SPHINGOSINE-1-PHOSPHATE SIGNALLING IN HUMAN RESISTANCE ARTERY FUNCTION IN HEART FAILURE

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Anatomically, regulation of total peripheral resistance (TPR) localizes to pre-capillary small arteries and arterioles ("resistance arteries"). Dynamic regulation of their resistance serves to regulate tissue perfusion and maintain constant flow over a wide pressure range (auto-regulatory window: 60 to 170mmHg). The most important mechanism regulating resistance artery tone and resistance is the intrinsic property of microvascular smooth muscle cells to adjust their contraction level to the amount of pressure sensed ("myogenic response"). In heart failure, increased myogenic responsiveness sustains elevated TPR, thereby stabilizing blood pressure and organ perfusion. This increase in myogenic responsiveness results from tumour necrosis factor-alpha (TNFα)-induced enhancement of sphingosine-1-phosphate (S1P)- a key regulator of the myogenic response. Although initially beneficial, increased TPR amplifies cardiac workload and exacerbates heart failure over time. This mechanism is a novel therapeutic target for heart failure. We aim to translate this mechanism from mice to patients. We hypothesize that S1P is critical for regulation of myogenic responsiveness in human resistance arteries, and arteries from heart failure patients have TNFα-dependent upregulated S1P signalling causing increased myogenic responsiveness. Using quantitative RT-PCR, we found expression of all S1P pathway components in arteries from patients with and without heart failure. Functional dependence on S1P for myogenic responsiveness was demonstrated using perfusion myography combined with chemical/genetic inhibition of S1P pathway components (Sphk1, CFTR). Severity of clinical heart failure is routinely determined by chart review. As functional data from human resistance arteries is sparse in the current literature, we first established that human mesenteric resistance arteries possess normal functional responses such as dose-dependent constriction to phenylephrine (logEC50 = -5.9±0.3), dilation to acetylcholine (10μmol/L), pressure-dependent vasoconstriction and constriction to S1P (logEC50 = -6.8±0.2). These arteries also have increased myogenic responses to exogenous S1P (10 nmol/L) or chemical inhibition of CFTR (1 nmol/L). Preliminary data confirm similar functional responses in skeletal muscle resistance arteries. These results show S1P modulates myogenic tone, suggesting a role for S1P signalling in human resistance arteries, translate key findings from mice, demonstrate the feasibility of testing these human vessel beds, and form a strong foundation for translating mechanisms from mouse models to human heart failure.

C9. GENERATION AND CHARACTERIZATION OF ERp44 (TXND4) KNOCKOUT/KNOCKIN MICE AND ZEBRAFISH KNOCK-OUT TO STUDY THE ROLE OF ERp44 Ca^{2+} SIGNALLING AND ER STRESS IN THE HEART

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Understanding the role of ER stress in cardiovascular disease is critical. ERp44 is a novel unfolded protein response (UPR)-induced endoplasmic reticulum (ER) protein that is widely expressed in a variety of tissues. ERp44 may be involved in multiple cellular functions including the inhibition of Ca^{2+} release by inactivating inositol 1,4,5-trisphosphate receptor1 (IP_3R1). Dysregulation of Ca^{2+} cycling in myocytes will activate ER stress and lead to cell death. The purpose of this study was to investigate the role of ERp44 in Ca^{2+} signaling and ER stress *in vivo* using ERp44 zebrafish and mice knockout (KO). I hypothesized that ERp44 plays important roles in ER stress involved in heart disease. Our results showed that there are severe phenotypic differences between the ERp44 KO compared to the wildtype (WT) mice. The ERp44 KO mice had low body weight and decreased activity. The survival rate of the KOs was extremely low (<4%), since majority of the mice died at E11.5. The ERp44 KO zebrafish had delayed embryonic development, pericardial edema, enlarged heart chambers, abnormal cardiac looping and slowed blood circulation. WT and heterozygous (HE) mice embryos were LacZ stained and a high expression of ERp44 was observed in early embryonic stages, however the expression declined as the embryo aged. Interestingly, our results indicated that ERp44 is strongly expressed in the heart during early stages; but the expression is localized to the endothelial and vasculature region in the adult heart. To further investigate the role of ERp44 in the heart, the neonatal cardiomyocytes and the zebrafish heart were also assessed for Ca^{2+} transient. Our results illustrated that there is a higher Ca^{2+} release amplitude in the ERp44 KOs and HE versus the WT. Therefore, it is clear that ERp44 plays a significant role in early embryonic stages and that ERp44 may be involved in negative regulation of Ca^{2+} transient in the heart.

C10. INTERROGATION OF THE FUNCTIONAL EXPRESSION OF SLC6A14 IN BRONCHIAL EPITHELIAL CELLS WITH WT AND F508DEL CFTR

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A recent genome-wide association study identified *SLC6A14* as a modifier gene for Cystic Fibrosis (CF). SLC6A14 is the only NaCl-dependent transporter of basic and neutral amino acids ($B^{0,+}$ system) and expression has been shown in intestinal and airway epithelial cells (Sloan,1999; Galietta, 1998). My objective is to study the functional expression of SLC6A14 and its possible interaction with CFTR using the CFBE41o- cell line overexpressing either WT or dF508 CFTR and primary human bronchial epithelial cells. Uptake using 1 μCi/ml [3H]-L-arg and increasing doses of L-arginine (L-arg) (20μM-500μM) follows Michaelis-Menten kinetics, with Km=90.9 μM (95%CI: 73.96-107.8) and Vmax=74.0 nmol/min/mg protein (95%CI: 69.86-78.14) in CFBE-WT, and a significantly lower Km= 68.1μM (95%CI: 55.7-80.55) (P=0.036) and higher Vmax =82.2 μmol/min/mg protein (95%CI: 78.14-86.19) (P=0.009) in CFBE-dF. Cellular uptake of 100μM Arg in the presence of Na^+ is similar in CFBE-WT (79.8±23 μM) and CFBE-dF (64.4±21 μM , P=0.34). Removal of Na^+ reduced L-arg uptake significantly in CFBE-dF cells (P<0.0001, n=15) indicating presence of system $B^{0,+}$ but results are inconclusive in CFBE-WT cells (P=0.23, n=6). However, alpha-methyltryptophan, an inhibitor of SLC6A14 (Karunakaran *et al.*, 2008), significantly reduced uptake of 100μM L-arg in both cell types (P<0.0001) arguing in favor of the presence of SLC6A14. Competitive inhibition experiments using L-lysine and L-leucine further suggested presence of systems y^+L and y^+ in CFBE cells. Therefore, in further studies primary bronchial epithelial cells were used to allow separation of apically and basolaterally localized L-arg amino acid transport systems. In healthy primary human airway cells the transepithelial L-arg transport was 1.97±0.16 nmol/cm^2 measured 30 minutes following apical addition of L-arg. L-arg transport was significantly reduced when apical Na^+ was removed (1.0 ±0.05 nmol/cm^2, P=0.026) suggesting a 50% contribution of SLC6A14 to L-arg transport. Future studies will investigate whether SLC6A14-mediated L-arg transport is increased in primary airway cells from CF patients.

C11. IDENTIFICATION OF NOVEL CARDIAC CELL PROTEIN MARKERS

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The heart is composed primarily of four resident cells types: contracting cardiomyocytes (CM), endothelial cells (EC), smooth muscle cells (SMC), and fibroblasts (FB). These cells have unique proteomes both in regards to the other constituent cell types and to the same cell types in other areas of the body. Protein expression changes in development and disease are thought to be associated with certain cell types, but the current knowledge of these proteomes hinders the connection of protein expression changes to the cell

source. Cationic silica beads were utilized to isolate plasma membrane protein enriched and depleted fractions from human CM, EC, and SMC, as well as human fetal ventricular and atrial cells. These fractions were analyzed by liquid chromatography tandem mass spectrometry via multidimensional protein identification technology, which identified 1266, 1268 and 946 proteins in the CM, EC and SMC cells respectively, of which 447, 503 and 80 proteins were found to be unique or enriched. Proteins identified were ranked based on: enrichment in a microarray in the heart and associated cell type; lack of previous cardiac annotation; presence in the fetal fractions; and enrichment in relation to the other cell types. According to this filtration strategy 56, 63 and 11 of the proteins were highly ranked. These proteins will be investigated further by RT-PCR, immunoblots, and confocal microscopy to identify both plasma membrane and cytosolic proteins that are specific to the cardiac constituent cells. Given that FB constitute a major cell type in the heart, work is also underway to perform a similar analysis of human FB. Together these data will to this study to form a more comprehensive view of cardiac proteins.

C12. MECHANISMS UNDERLYING ATRIAL FIBRILLATION IN CAV1.3 KNOCK-OUT MICE

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Atrial fibrillation (AF) is the most common sustained supraventricular arrhythmia and is a prominent cause of thromboembolic complications associated with impaired cardiac performance. Transgenic mice lacking the L-Type Ca^{2+} a1D subunit ($Ca_v1.3$ -/-) are more susceptible to atrial fibrillation (AF), with diminished calcium (Ca^{2+}) currents (I_{Ca-L}) and reduced sarcoplasmic reticulum Ca^{2+} stores. Since the probability of primary sparks significantly increases under conditions of reduced SR load and reduced I_{Ca-L} density which has been shown to result in calcium discordinance, formation of Ca^{2+} alternans, and action potential duration (APD) alternans, all of which increase the likelihood of electrical reentry, we hypothesized that Cav1.3-/- mice are more susceptible to AF due primarily to Ca2+ alternans. Using a rapid stimulation protocol on isolated atria, 73% $Ca_v1.3$-/- mice underwent sustained AF with significantly longer AF durations compared to $Ca_v1.3$+/+ mice. Since Ca2+ alternans increase the complexity of AF, dynamic field recordings of AF and optical images acquired during AF were subjected to frequency domain analysis. In agreement with our hypothesis, these studies revealed significantly greater complexity of AF in $Ca_v1.3$-/-, as evident by dynamic instability and tissue heterogeneity of dominant frequencies. Furthermore, $Ca_v1.3$-/- atrial myocytes isolated from the left appendage showed a significantly greater susceptibility to calcium alternans with a large degree of discordinance when paced at 6Hz and 7Hz. Interestingly, optical mapping of $Ca_v1.3$-/- isolated atria showed a significant reduction in epicardial conduction velocity. Although perturbed Ca2+ handling, as shown here, does not directly influence conduction velocity, increased collagen deposition in the atria can lead to conduction slowing. Consistent with this, atrial collagen content was found to be significantly elevated in Cav1.3-/- mice with a concomitant p-wave duration prolongation. In conclusion, it appears that the loss of Cav1.3 directly leads to perturbed Ca2+ handling, increasing the likelihood of Ca2+ alternans, but also leads to structural remodeling, both of which ultimately increase the likelihood of electrical reentry in the atria.

C13. IDENTIFYING THE ROLE OF A NOVEL ADAPTOR PROTEIN, XB130, IN TUMORIGENESIS

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XB130 is a novel adaptor protein that regulates cell proliferation and survival through modulating PI3K/AKT pathway. Previous studies have indicated that XB130 plays an important role in promoting cell cycle progression, motility and invasion, and in reducing cell death in various human thyroid and lung cancer cells. However, the function of XB130 in *in vivo* tumorigenesis remains unknown. We hypothesized that XB130 may play a significant role in tumorigenesis *in vivo* through modulating cell cycle progression and inflammatory response. To initiate the study, XB130 knockout (KO) mice were generated. The dorsal area of 8 week-old KO mice and their littermate wild-type (WT) controls were topically treated with a single application of a tumor initiator mutagen, DMBA (25nM), followed by repeated semi-week application of a tumor promoter carcinogen, TPA (40nM). Both groups began to develop tumors 7-8 weeks after DMBA treatment. At week 33, mice were sacrificed to harvest both tumor and hyperplastic lesions. Histopathological evaluation using H&E staining indicated that the tumor lesions of week 33 could be categorized into three subtypes: dysplasia, verrocous dysplasia, and keratoacanthoma. Two groups displayed no statistical differences in the frequency of each subtype. However, the tumour incidence was significantly lower in the KO mice than the WT mice ($p<0.05$) in early weeks of tumor promotion (week 10&13), but higher in the later weeks (week 23-33). Moreover, the WT group had a significantly higher number of tumors smaller than 2mm in diameter in early weeks, whereas the KO group had a higher number of 2-6mm-sized tumors later weeks. Additionally, the KO mice were more susceptible to TPA-induced skin inflammation. Therefore, we conclude that XB130 has a controversial role of a tumor promoter and a tumor suppressor in early and late phase of tumorigenesis, respectively, and that further studies are necessary to elucidate its underlying mechanism.

C14. EFFECTS OF GLP-1 ON HUMAN MEGAKARYOCYTES AND PLATELETS

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Studies of glycemic control in diabetics suggest that glucagon-like peptide-1 (GLP-1)-targeted drugs reduce the risk of cardiovascular events over 6-12 months. Mechanisms underlying this unexpectedly rapid effect are not known. We hypothesize that GLP-1 may exert anti-thrombotic or anti-platelet effects. To test this, freshly isolated human platelets and a megakaryocyte cell line (MEG-01) were studied. RT-PCR on RNA from MEG-01 cells followed by cloning and sequencing revealed the expression of full-length GLP-1R mRNA. Validation of this finding involved qRT-PCR with established primers on MEG-01 cDNA. A dose-dependent increase in intracellular cAMP in MEG-01 cells ($2x10^5$/ml) treated with GLP-1 gives further evidence supporting the likelihood of a functional adenylate cyclase-coupled GLP-1R ($n=3$, $p<0.01$). As we were unable to detect dipeptidyl peptidase-4 (DPP-4) activity in platelets or MEG-01 cells ($n=3$, $p<0.01$), we believe cAMP effects are likely receptor-mediated. To test whether GLP-1 could exert a biological effect on platelet function, freshly isolated gel-filtered human platelets ($3x10^8$/ml) were incubated with GLP-1 for 15 min and stimulated to aggregate with 0.125U thrombin. GLP-1 (1nM) delayed platelet aggregation by ~60% 1 minute after activation ($n=3$, $p<0.05$). The GLP-1R agonist exenatide also delayed aggregation in a dose dependent manner after platelets were stimulated with 0.5U thrombin. Together, these results suggest that human platelets and megakaryocytes express a functional GLP-1R which, when activated, inhibits platelet aggregation.

C15. DISCOVERY PROTEOMICS STRATEGY IDENTIFIES HYPOXIA-INDUCED CHANGES IN THE CARDIAC FIBROBLAST EXOSOME

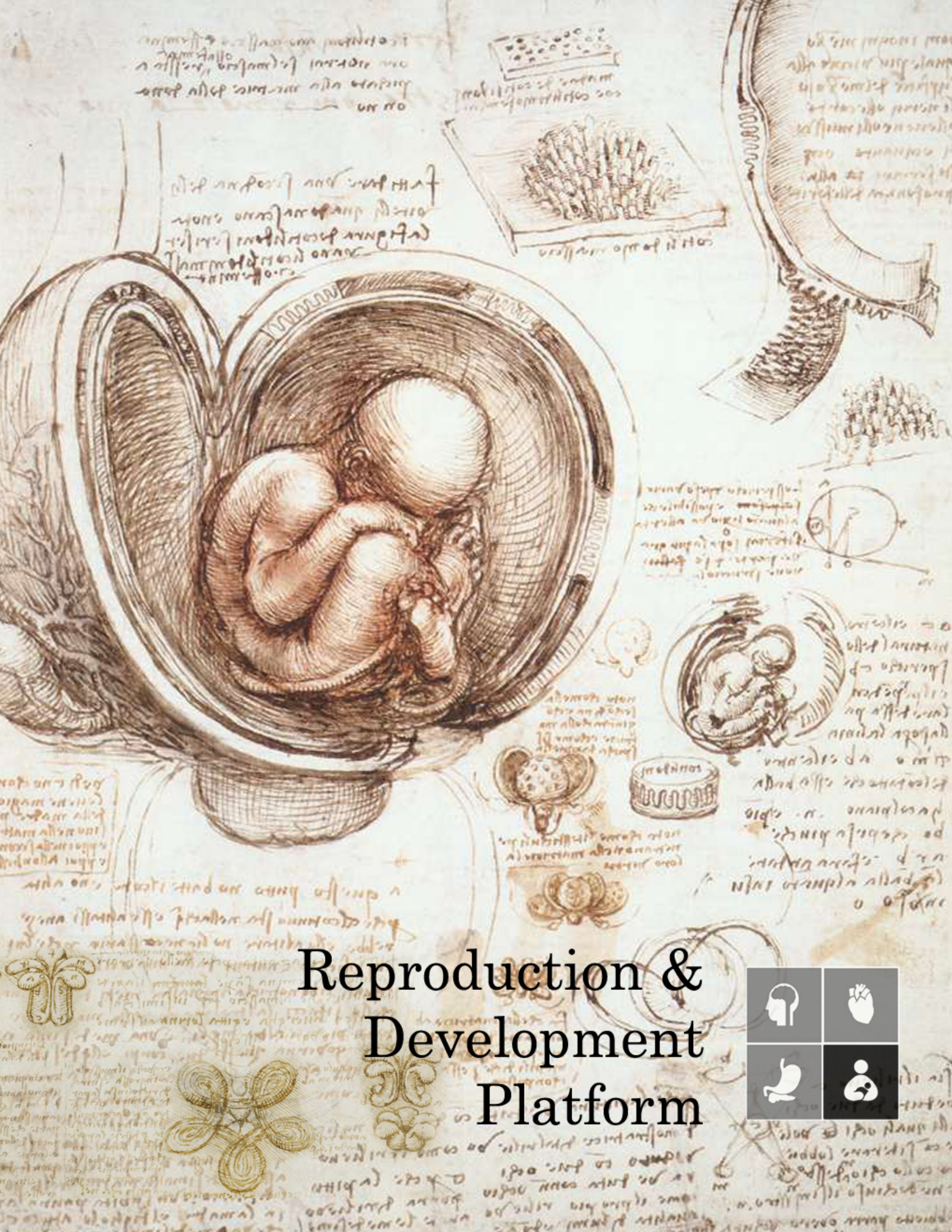
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The cardiac fibroblast (CF) plays a central role in the physiological outcomes of heart failure after ischemia and cardiac fibrosis. With the CF being the largest stationary cell population in the heart, number-wise, it is important to understand their communication with other cell types in the heart. The exosome is a secreted microvesicle of 10-100 nm in diameter released via exocytosis. Exosomes have shown to be a cardioprotective agent *in vitro* and affected in the cardiac stress response. Proteomic investigations may provide insight on how the exosome is altered in the stress response. Mouse neonatal CF were cultured up to P1 and maintained in 21% O_2. CF were then subjected to either 21% (normoxic) or 2% (hypoxic) O_2 for 24 hr in serum-free media. Conditioned media was cleared via differential centrifugation at 2000 × g and 10 000 × g before concentration. Exosomes were then isolated via ultracentrifugation at 120 000 × g. Exosomes were characterized by electron microscopy, nanoparticle tracking analysis (NTA), and Western blot analysis for exosome markers. Mass spectrometry data was acquired using a multi-dimensional protein identification technology (MuDPIT) approach from 4 biological replicates analyzed in duplicate. Data was searched using an automated protein database search algorithm through MaxQuant. Electron microscopy and NTA showed CF exosomes range between 10 – 100 nm in diameter. MuDPIT analysis identified 1601 proteins which included exosome markers such as 14-3-3 proteins and tetraspanins. Initial bioinformatic strategies based on peptide counts have identified 125 proteins that are upregulated or exclusive to the hypoxic CF exosome compared to normoxic CF exosomes. Gene ontology (GO) analysis of this subset of proteins shows enrichment for GO terms that include mitochondrial membrane, transport, and transit peptide. Further bioinformatic analysis of the data is currently being undertaken in order better understand these subproteome differences.

C16. EVALUATION OF HUMAN UMBILICAL CORD PERIVASCULAR CELLS IN CARDIAC REGENERATION

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Multipotent mesenchymal stem cells isolated from various tissues have been investigated in the context of cell therapy for the injured myocardium. In this study, we isolated multipotent cells from the perivascular region of first trimester and term human umbilical cords (FTM-PVCs and TERM-PVCs, respectively) and evaluated their capacity to differentiate into cells with cardiomyocyte features. Furthermore we demonstrate that PVCs possess the capacity to regulate the function and promote the survival of cardiomyocytes following hypoxia/reperfusion injury in vitro. Both FTM-PVCs and TERM-PVCs underwent morphological changes resembling cardiomyocytes upon co-culture with either rat or human cardiomyocytes. Immunocytochemical and flow cytometrical analysis revealed that the proportion of α-SA and cTnT positive cells was significantly increased in co-culture conditions compared to the control medium, and to comparable extents in FTM-PVCs and TERM-PVCs. Cardiomyocytes undergoing in vitro ischemia/reperfusion show signs of oxidative and glucose deprivation injury, that appears to be partially rescued by co-cultured PVCs. Our study suggests that umbilical cord-derived perivascular cells have cardiomyogenic potential, but that optimization of our induction conditions or an in vivo environment are required to generate functional cardiomyocytes. We argue that clinical relevance of post-infarct application of PVCs can also be in their capacity to promote the regeneration of cardiomyocytes through secreted factors and cell-to-cell contact communication.



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TRANSFORMING GROWTH FACTOR-β1 IS A POTENT ACTIVATOR OF DRUG TRANSPORT IN THE FETAL BLOOD-BRAIN BARRIER (BBB)

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The developing brain is protected from a range of xenobiotics by multidrug resistance transporter, P-glycoprotein (P-gp). P-gp expression increases rapidly in the fetal brain BBB in late gestation. During this period, TGF-β1 is released by astrocytes in the developing brain. TGF-β1 has been shown to modulate P-gp activity in adult cell-types. However, little is known about how TGF-β1 affects P-gp in brain endothelial cell (BECs) in late gestation, when the brain is most vulnerable to teratogens. The objectives of this study were to determine the effect of TGF-β1 on P-gp expression and activity in the BBB at critical phases of brain development, and to determine the signaling pathways involved. We hypothesized that TGF-β1 will increase P-gp expression and activity but that the magnitude of effect will change with age. BECs were isolated from gestational day(GD)40, GD50, GD65 and postnatal day(PND)14 guinea pigs (n=6-8). At confluence, BECs were treated with TGF-β1(0.001-10ng/ml) for 2-24h. To determine the signaling pathways involved, BECs were treated with ALK1 and ALK5 antagonists. P-gp activity was assessed using calcein-AM assay and *abcb1* mRNA (encodes P-gp) by RT-PCR. Expression of TGF-β receptors were quantified. TGF-β1 dose-dependently increased *abcb1* mRNA and P-gp activity in BECs derived at all ages. However, GD40 & GD50 BECs were more responsive than PND14 BECs. Betaglycan, which decreases responsiveness to TGF-β1, increased with age, correlating with the blunted response to TGF-1 in PND14 BECs. Analysis of signaling pathways involved revealed importance of the ALK1 pathway. TGF-β1 is a potent modulator of *abcb1* expression and P-gp activity in the fetal BBB, with most pronounced at earlier stages of development. We have also identified the specific signaling pathways involved. These results indicate that TGF-β1 released from astrocytes upregulate P-gp at the BBB. However, aberrations in TGF-β1 levels in BECs, resulting from altered glial differentiation or fetal plasma TGF-β1, may lead to substantial changes in fetal brain exposure to xenobiotics and other P-gp substrates.

CHARACTERIZING THE GENE EXPRESSION PATTERN OF MYOMETRIAL MMPS AND TIMPS THROUGHOUT PREGNANCY, LABOUR AND POST-PARTUM

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It has been widely accepted that term labour is an inflammatory process involving the influx of activated immune cells that can produce extracellular matrix (ECM)-degrading proteolytic enzymes Matrix Metalloproteases (MMPs). We hypothesize that during late pregnancy there is an increase in myometrial MMPs' and a decrease in their tissue protein inhibitors (TIMPs') expression/activity to promote myometrial inflammation during labour and following post-partum uterine involution. It has also been reported that *in vivo* mechanical stretch of the uterine wall by the growing fetus modulates expression of ECM components. We hypothesized therefore that mechanical stretch can regulate a coordinated MMPs' and TIMPs' gene expression triggering ECM remodeling during late pregnancy and labour. To test this hypothesis we measured the expression of MMP and TIMP genes using two *in vivo* rat models of bilateral and unilateral pregnancies. Myometrial tissues from bilateral pregnant rats and from gravid and non-gravid (empty) horns of unilaterally pregnant rats were collected throughout gestation, labour (d23L) and post-partum (PP). RNA was extracted, reverse transcribed and subjected to real-time RT-PCR analysis for MMP1-3, 7-14, TIMP1-4. MMP 1b,2,8-14 expression was elevated one day (d) prior to labour (d22), decreased during labour and was significantly induced again during PP period ($p<0.05$). Expression of MMP7 was transiently up-regulated on d22 (3000-fold increase), culminated during labour (5000-fold increase, $p<0.001$) and decreased during PP period. TIMP1,2 expression was elevated at mid-gestation (d12) and declines towards labour and post-partum ($p<0.01$). In contrast, TIMP3,4 mRNA levels were increased d4PP ($p<0.05$ compared to d23L). A similar trend of all MMPs' myometrial gene expression was observed in the gravid uterine horn, but not in the empty horn of unilaterally pregnant rats. Our data are consistent with MMPs and TIMPs playing a role in the remodeling of myometrial tissue to prepare the uterus for labour contractions and post-partum involution.

MODULATION OF DNA METHYLATION AND PHENOTYPIC SWITCHING IN SMOOTH MUSCLE CELLS BY DAMAGED MATRIX

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In partial bladder outlet obstruction, stretch, hypoxia and damaged matrix (DNC) induces hyper-proliferation and de-differentiation in bladder Smooth Muscle Cells (BSMCs). Previously, DNC induced hyper-proliferation in BSMC was not reverted upon a return to normal matrix. Therefore, epigenetic change may underlie the persistent fibroproliferative phenotype. We examined the dependency of fibroproliferation and SMC phenotype on DNA methyltransferase (DNMT) activity. Primary cultures of rat and human BSMC were plated on either native collagen (NC) or heat-denatured NC (DNC). Cells were fixed, stained for DNMT3A and α-Smooth muscle actin (α-SMA). Intensities and cell numbers were analysed on ImageJ. Illumina 450K array of CpG sites was performed on bisulfite converted DNA from human BSMC on DNC vs. NC. While BSMCs on NC expressed cytoplasmic DNMT3A, DNC exposure significantly translocated DNMT3A into the nucleus. DNC also induced expression of α-SMA . Aza-cytidine suppressed hyper-proliferation of BSMCs cultured on DNC without affecting basal proliferation on NC. After rigorous multiple testing, 7 genes had significant changes in methylation status in the Illumina 450K array. Matrix exquisitely regulates DNMT3A localization and expression, and influences differentiation in BSMCs exposed to denatured matrix. The translocation of DNMT3A, a de novo methyltransferase, suggests that damaged matrix induces alterations in epigenetic machinery of BSMC.

Repro: Posters

R1. STRETCH-INDUCED MYOMETRIAL CYTOKINES ENHANCED PERIPHERAL NEUTROPHIL RECRUITMENT VIA ENDOTHELIAL ACTIVATION

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Spontaneous labour at term is associated with increased cytokine production, endothelial cell adhesion molecule expression (CAM) and leukocyte invasion in the myometrium. We previously demonstrated an association between mechanical stretch and release of pro-inflammatory cytokines by human myometrial cells *in vitro*. This suggests a potential correlation between uterine stretch and peripheral leukocyte recruitment in the myometrium during late gestation, attributed to the elevated local cytokine levels. We hypothesize that stretch-induced cytokines facilitate peripheral leukocyte transmigration into the myometrium via upregulation of endothelial CAMs and enhancement of the local chemotactic environment. Conditioned media (SCM) was collected after 24 hours of static stretch of human myometrial cells. E-selectin (SELE), ICAM-1, VCAM-1, PECAM-1 gene and protein expressions in human uterine myometrial microvascular endothelial cell (UtMVEC-Myo, Lonza) were investigated after stimulation with SCM for 4 or 6 hours by Real-Time PCR and flow cytometry. Ahesion and transendothelial migration assays were executed using primary human neutrophils with UtMVEC-Myo seeded onto gelatin-coated 96-well microplate or 3-μm inserts with or without neutralizing Abs to chemokine receptors (10 μg/ml) or broad-spectrum chemokine inhibitor (BSCI, 2nM). Stimulation with SCM significantly increased endothelial *SELE*, *ICAM1* and *VCAM1* mRNA expressions, with corresponding increase in the number of VCAM1+ and SELE+ endothelial cells (2.27% to 9.43% and 0.77% to 6.44% respectively), as well as an increase in ICAM1 mean fluorescence intensity value (52.98 to 79.61) when compared to control. SCM significantly enhanced neutrophil adhesion to UtMVEC-Myo by 1.97-fold. It also increased neutrophil transmigration, an effect that was attenuated upon neutralization of CXCR1 and CXCR2. Preliminary results showed that administration of BSCI decreased neutrophil transmigration to a level similar to that of negative control. Our results demonstrated the role of stretch-induced cytokines in neutrophil recruitment into the myometrium. The presence of immune cells in the myometrium during late gestation creates a localized inflammation, which potentiates uterine activation in preparation for labour contraction and further postpartum involution process.

R2. EFFECT OF UNILATERAL OVARIECTOMY AND FETECTOMY ON MURINE PLACENTAL ANGIOGENESIS IN LATE GESTATION

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In late gestation, the rate of fetal growth increases rapidly, requiring matched increases in nutrient and oxygen delivery. Although there is little change in placental weight at this stage, there is a large increase in placental capillaries resulting in an increase in the surface area for maternal-fetal exchange. The angiogenic mechanisms regulating this increase are not known. The objective of this study is to understand the main mediator of fetoplacental angiogenesis by assessing the changes in placental vascularity upon removal of fetal factors or with reduced maternal ovarian factors. Pregnant mice underwent surgery on gestational day 15.5 (gd 15.5; 4 days before term). Two fetectomies, via fetal cardiac injection of a lethal dose of KCl, and either a unilateral maternal ovariectomy or sham operation were performed. 48 hours later, the mice were euthanized and blood samples were collected to measure circulating hormone levels. The remaining ovary, placentas, and fetuses were weighed. Placentas were immunofluorescently stained for CD34 and Ki67 to assess vascularity and endothelial cell proliferation in the labyrinth. Apoptosis will also be evaluated using TUNEL. Ovariectomy caused no apparent contralateral ovarian hypertrophy at E17.5 or significant differences in fetal weight. There was also no significant difference in placental weight. Fetectomy significantly reduced fetal weight of injected fetuses (as expected), but placental weight did not differ. Nevertheless, we expect morphological differences in the placenta between the ovariectomy group and sham, as well as between fetectomy and control groups. We anticipate that the reduction in fetal factors will result in a reduced labyrinth, with greater apoptosis and less proliferation of endothelial cells. While reduced maternal ovarian factors in circulation will result in less apoptosis and an increased number of proliferating endothelial cells in the labyrinth. Next, we will assess changes in placental expression of angiogenic factors believed to be involved in fetoplacental angiogenesis.

R3. ALTERED EXPRESSION OF INFLAMMATION-ASSOCIATED GENES IN BOVINE OVIDUCTAL CELLS IN RESPONSE TO FOLLICULAR FLUID EXPOSURE

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Evidence indicates that high-grade serous ovarian carcinoma originates from lesions within the distal fallopian tube epithelium. Events associated with increased ovulation, a micro-inflammatory event of which delayed resolution may contribute to malignant transformation, increase the risk of this carcinoma. Our previous studies indicate that fallopian tube epithelial cells from carriers of germline mutations in breast cancer susceptibility genes exhibit a pro-inflammatory gene expression signature during the luteal phase, suggesting that delayed resolution of post-ovulatory inflammatory signaling may contribute to predisposition to this ovarian cancer histotype. To determine if exposure of tubal epithelial cells to peri-ovulatory follicular fluid alters expression of inflammation-associated genes, we developed an *ex vivo* culture system using bovine oviductal epithelial cells. Oviductal cells grown on collagen IV-coated transwell membranes assumed a cobblestone appearance consistent with an epithelial sheet. Immunocytochemistry for FoxJ1 and Pax8 indicated both ciliated and secretory epithelial cells were maintained in the cultures. Oviductal cells in primary culture were exposed to human follicular fluid or culture medium for 24 h following which total cellular RNA was extracted at various time points. Expression of genes associated with inflammation was determined by quantitative real-time RT-PCR. Exposure to follicular fluid transiently altered the expression of seven of the nine genes examined. This study demonstrates that peri-ovulatory follicular fluid can act directly upon oviductal epithelial cells to alter gene expression that might contribute to early carcinogenic events. Furthermore, these findings illustrate the potential use of bovine oviductal cells to study signaling events implicated in ovarian carcinogenesis.

R4. ANALYSIS OF THE DIRECT REGULATION OF REPRODUCTIVE-ASSOCIATED GENES IN HYPOTHALAMIC KISSPEPTIN-EXPRESSING NEURONAL CELL MODELS

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Fertility is a complex and highly regulated process dependent on the orchestration of hypothalamic neuropeptides and peripheral hormones. Signals converge on gonadotropin-releasing hormone (GnRH) neurons, positioned at the pinnacle of the hypothalamic-pituitary-gonadal (HPG) axis.

Kisspeptin (Kiss) and its receptor, GPR54, have emerged as fundamental gatekeepers of reproduction, acting centrally upstream of GnRH neurons. It is well established that Kiss neurons express estrogen receptors, and estradiol-mediated regulation of these neurons is nuclei-specific. Further, subpopulations of Kiss neurons have been found to express GPR54 and the gonadotropin-inhibitory hormone (GnIH) receptor, GPR147, suggesting an additional level of regulation in Kiss neurons. Currently, *in vitro* studies focusing on the regulation of key genes expressed within hypothalamic Kiss neurons are limited. To address this issue, we have generated immortalized, clonal cell lines derived from rodent hypothalamic primary culture. We have identified two cell lines, mHypoA-50 and mHypoA-55, which exhibit endogenous Kiss expression, as well as GPR54, GPR147, and estrogen receptors (ERα, ERβ, GPR30). Using qPCR, we report an induction of GPR54, ERα and ERβ mRNA expression with estradiol treatment at 4 h and 24 h in both cell lines. Interestingly, while Kiss-10 treatment induces GPR54, ERα and ERβ mRNA expression in the mHypoA-55 cell line, no change was measured in the mHypoA-50 cell line. Following GnIH treatment, in the mHypoA-50 neurons, we report a significant decrease in Kiss1 and GPR54 mRNA expression, whereas in the mHypoA-55 neurons, there was suppression of Kiss1 expression alone. Together these results highlight differences between populations of hypothalamic Kiss neurons. Current studies using ER agonists and antagonists, and Western blot analyses, are being used to delineate the direct estradiol and Kiss-10-mediated mechanisms controlling transcription of the genes studied. These studies will help us to define the mechanisms by which different populations of Kiss neurons are regulated and modulate reproductive function.

R5. REGULATION OF MULTIDRUG RESISTANCE IN TERM HUMAN PLACENTAL EXPLANTS IN CULTURE

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The placenta contains efflux transporters, including P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), that limit the passage of xenobiotics, certain hormones and nutrients from the maternal to the fetal circulation. While studies have been conducted in animals, there are limited models available to investigate placental multidrug resistance in human tissue. The objective of this study was to investigate P-gp and BCRP expression in the human placenta using an *in vitro* culture model. As previous studies have demonstrated syncytiotrophoblast regeneration in these culture conditions, we hypothesized that the regenerated syncytiotrophoblast layer would express high levels of P-gp and BCRP. Term healthy placentae (n=6) were collected and cultured for a 6-day period. Culture medium was collected every 24h to measure lactate dehydrogenase (LDH; explant viability) and human chorionic gonadotropin (hCG; syncytiotrophoblast function). P-gp (encoded by *ABCB1*) and BCRP (encoded by *ABCG2*) protein and mRNA expression were measured before and after culture in the same placentae using western blot and qRT-PCR. We also measured the protein expression of HIF1α, an established key regulator of P-gp, using western blot. Cultured explants had reduced hCG levels by day 2 and increased levels following day 3, indicative of syncytiotrophoblast shedding and regeneration. There was a very significant decrease in P-gp protein and *ABCB1* mRNA expression in cultured compared to pre-cultured tissue, however there was no change in BCRP protein or *ABCG2* mRNA expression. There was a significant decrease in HIF1α protein in cultured explants compared to pre-cultured tissue; this was strongly correlated with P-gp expression. We have identified profound differences in the expression of placental P-gp and BCRP transporters following *in vitro* culture. Interestingly, the regenerated syncytiotrophoblast layer expresses extremely low levels of P-gp, but 'normal' pre-culture levels of BCRP. The reduction in P-gp correlated closely with that of HIF1α, which is known to positively regulate P-gp in other cell types. Understanding the potential role of HIF1α in the regulation of P-gp is important as emerging evidence indicates these proteins are aberrantly expressed in pathologies of pregnancy, including pre-eclampsia and IUGR.

R6. SINGLE COURSE ANTENATAL GLUCOCORTICOID HAS MULTIGENERATIONAL EFFECTS ON GROWTH, ENDOCRINE FUNCTION AND BEHAVIOR

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Antenatal synthetic glucocorticoid (sGC) exposure can permanently 'program' metabolic, cardiovascular and neurologic function in offspring. We have recently shown that multiple course antenatal sGC treatment leads to profound effects on hypothalamic-pituitary-adrenal (HPA) function and behavior in second generation (F_2) offspring. Currently, single course treatment represents the 'gold-standard' of care. In the present study, we hypothesized that single course sGC therapy would significantly affect growth, HPA function and behavior in juvenile F_2 offspring. Pregnant guinea pigs were subcutaneously treated with betamethasone (Beta; 1mg/kg; n=12) or saline (n=11) at 75% of gestation (term ~69 days). Adult F_1 females were mated with control males to produce F_2 offspring; there was no manipulation during pregnancy. Anthropometric measures were taken at birth and on postnatal day (PND) 20. F_2 offspring were tested in an open-field (30 min) to assess locomotor activity on PND19 and 24. Attention was assessed by prepulse inhibition (PPI) testing on PND23. HPA function was assessed under activated (PND19 & 24) and basal conditions (PND26). Single course sGC exposure significantly reduced birth weight ($P<0.01$) and abdominal circumference ($P<0.05$) in F_2 males. At PND19 and 24, there were no significant effects of sGC treatment on locomotor activity. Beta female and male offspring mounted more rapid HPA responses to stress ($P<0.05$). At PND26, only Beta F_2 males demonstrated a diurnal change in basal cortisol ($P<0.05$). There was no effect of sGC on attention in either sex, but the startle response was reduced in Beta males ($P<0.05$). Prenatal treatment with single course sGC leads to multigenerational effects on growth, behavior and HPA function. We are currently investigating the molecular mechanisms that underlie multigenerational programming by sGC. Given that approximately 10% of all children in the developed world have been prenatally exposed to sGC, it is critical that we understand the long-term implications of such therapy.

R7. NOVEL MECHANISMS OF HIF-1α REGULATION IN GESTATIONAL DIABETES

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The transcription factor, HIF-1 α (Hypoxia-inducible factor 1α) is a master regulator of the cell's response to hypoxia. Its stability is tightly regulated by the dioxygenase prolyl hydroxylases that target it for normoxic proteasomal degradation. We have previously reported significantly elevated levels of HIF-1 α as well as altered expression of the key prolyl hydroxylase, PHD2 in preeclamptic placentae relative to controls. It is well established that women affected by Gestational Diabetes Mellitus (GDM) are at a higher risk of developing preeclampsia. Hence, our main objective is to investigate the mechanisms regulating HIF-1α stability as well as the involvement of PHDs in this disease. Western Blot (WB) and quantitative Polymerase Chain Reaction (qPCR) analyses were used to characterize HIF-1α and PHD2 protein and mRNA expression in GDM placentae. To assess the effects of hyperglycemia, choriocarcinoma JEG-3 cells maintained at 3% and 20% O_2 were treated with 10 mM and 25 mM glucose for 24 hours followed by WB and qPCR for HIF-1α and PHD2. Our results revealed the presence of an additional band for PHD2 (at ~ 45 kDa) primarily in GDM placentae. *In vitro* studies suggest an increase in this unidentified band in PHD2 following glucose treatment in both 3% and 20% O_2. Conversely, hyperglycemia had a dichotomous effect on HIF-1α in different oxygen tension conditions whereby hyperglycemia promoted HIF-1α stabilization in 20% O_2 and destabilization in 3% O_2. In conclusion, our data shows that HIF-1α and its key regulator are altered in GDM and that this is in part dependent upon high glucose levels typical of this pathology. We postulate that the shift in PHD2 banding pattern indicates

exclusive post-translational modification of the enzyme in GDM. A better understanding of the interplay between glucose and oxygen axes will help elucidate the distinctive molecular signature of GDM.

R8. EXPRESSION OF VEPH1 DURING MOUSE EMBRYO DEVELOPMENT AND IN ADULT TISSUES

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Ventricular zone-expressed PH domain protein (Veph1) was discovered in the mouse as having an important function in the development of the central nervous system. Literature regarding the function of this protein and tissue expression is limited. Veph1 may be a novel disruptor of FoxO signaling as its Drosophila orthologue Melted interacts with and inhibits mediators of this cascade. Our lab has established an inhibitory effect of Veph1 on SMAD-dependent TGF-β signaling. Furthermore, we have identified an effect of Veph1 expression on other signaling pathways in human ovarian cancer cells that are involved in development and tumorigenesis. Two mRNA variants of Veph1 have been described in mice, one encoding the full-length protein (Veph1A) and another predicted to encode the C-terminal portion of the protein (Veph1B), including the PH domain. The objective of this study was to establish the expression of mouse Veph1 variants in embryonic and adult mouse tissues. Total RNA and protein were extracted from murine embryos (E9.5-18.5) and various adult tissues and were analyzed using RT-PCR and Western blot analysis, respectively. Localization of Veph1 protein was visualized by immunohistochemistry. Veph1A was expressed from embryonic day 10.5 through to E18.5, whereas Veph1B expression was not detected beyond E17.5. Veph1A RNA and protein were expressed in the ovary, oviduct, epididymis, testis, lung, brain, kidney, and eye but were not detected in the uterus, visceral fat, prostate, seminal vesicle, heart, liver, spleen or skeletal muscle. Veph1B expression was not detected in any adult tissue examined. Immunohistochemical staining indicated staining was predominantly restricted to epithelial cells. These data demonstrate that Veph1 expression, while restricted, is more widespread than originally thought. Expression of Veph1B transcripts is restricted to embryo development suggestive of differential effects of these two isoforms. Future investigation regarding the specific role of Veph1 proteins in signaling cascades within these tissues is warranted.

R9. DESIGNING AN IN VITRO MODEL OF KIDNEY DIFFERENTIATION AND A CO-CULTURE SYSTEM WITH DECELLULARIZED MATRICES

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Controlled differentiation of human and mouse embryonic stem cells lines have been used to produce cells of various lineages. Our goal is to design a differentiation protocol using various combinations and concentrations of growth factors that we can apply to both human and mouse ES cells in order to direct cells to the kidney lineage. Furthermore, we also co-cultured differentiated and undifferentiated stem cells on adult decellularized kidney matrices to determine interactions between differentiated and undifferentiated cells and a decellularized kidney matrix. We hypothesize that embryonic stem cells (ES) can efficiently be differentiated into kidney precursor cells through the addition of growth factors including members of the TGF-β superfamily and fibroblast growth factors. We are currently growing human and mouse embryonic stem cell lines to differentiate into metanephric mesenchyme and ureteric bud cell populations. Embryonic stem cells have been grown on collage type IV and gelatin coated plates and cell culture inserts. We have been able to design a protocol that induces an efficient population of intermediate positive cells (as confirmed by the expression of *Pax-2*). This involves growing cells in mesoderm differentiation medium for 2 days followed by 2 days in intermediate mesoderm differentiation medium. We have also been able to design a preliminary metanephric mesenchyme and ureteric bud differentiation medium and have achieved expression of *Six-2* and *Cytokeratin*, (markers of metanephric mesenchyme and ureteric bud, respectively). We determined that the decellularized kidney matrix is able to support and direct differentiation of undifferentiated stem cells and therefore possesses bioreactive properties. Our goal is to use this protocol along with co-culturing techniques to better understand kidney development and develop cell-based therapies for the treatment of kidney disease.

R10. DIFFERENTIAL EFFECTS OF HEPARIN ON PREECLAMPSIA PATIENTS- SUBCLASSFICATION OF PREECLAMPSIA

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Preeclampsia (PE) is a life threatening disorder of pregnancy, caused by placental insufficiency, and characterized by multi-organ dysfunction, hypertension, proteinuria and fluid retention. At the molecular level PE can be identified by excess anti-angiogenic factors, sFlt1 and sENG in maternal circulation, *but not in all patients*. Although low dose aspirin and heparin bolus may help some women, *no standard therapy has been developed that can treat all PE*. The assessment of PE patients as a single disease has likely led to failure of biomarker and therapy discovery. Under the hypothesis that PE is multi-factorial with different etiopathologies, we have modeled microarray data and identified three subgroups of PE patients with distinct expression signatures relating to angiogenesis, hormone production and chronic hypertension superimposed PE. Given these diverse molecular subgroups we now hypothesize that heparin will cause differential effects on morphology, gene expression and downstream signaling in placenta, which could be used to identify subtypes of PE patients that will respond to treatment. I will assess the changes in signaling in placental villous ex-plants treated with heparin and changes in gene expression and cell signaling in PE patients. First trimester placental explant culture conditions have been optimized and growth in RPMI media resulted in significantly reduced cell death ($p < 0.05$). Next, first trimester placenta will be grown in serum from PE women +/- heparin and assessed for differences in morphology and gene expression. Anti-phosphopeptide antibody arrays have been conducted in 32 patients with PE, chronic hypertension, and preterm controls to identify significant changes in downstream signaling events. Future experiments include microarray/proteomic analysis of PE placenta samples +/- heparin to determine changes in gene expression.

R11. EXAMINING THE EFFECTS OF HEPARIN TREATMENT ON CELL PROLIFERATION IN THE MOUSE PLACENTA: IMPLICATIONS FOR NOVEL THERAPEUTIC APPROACHES FOR PREECLAMPSIA

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Pre-eclampsia (PE) is a life threatening disorder of pregnancy. In PE, there are sites of infarction on the placenta attributed to the malformation of the placental villi. The maternal-fetal interface exchange surface is formed by a layer of syncytiotrophoblast (SCT) cells. Furthermore, there is a cytotrophoblast (VCT) cell layer situated under the SCT cell layer. The VCT cells are capable of undergoing symmetric divisions (to maintain the VCT cell pool), or terminal differentiation and fusion (to form the SCT cell layer). It is suspected that PE is associated with an altered balance of VCT division and differentiation. Being essential for maintaining mouse trophoblast stem cells, we suspect that heparin restores the altered balance in the VCT cell layer in PE. Pregnant CD-1 mice were injected with low molecular weight heparin (LMWH) starting from E9.5 until sacrifice at E12.5 or E14.5. In vivo BrdU injection was carried out to label placental proliferative cells. Plasma LMWH and sFLT1 levels were measured. The weight of the fetuses and placentas were recorded, and placental tissue was collected for mRNA or

immunohistochemistry (IHC). BrdU IHC was carried out to visualize proliferative cells. Proliferative trophoblast cells were visualized by PHH3 and cytokeratin 18 IHC double staining. Despite a significant rise in plasma LMWH levels (n=5, <2.5 fold increase, p<0.0001), There was no significant difference between the mice with respect to plasma sFLT1 levels. Moreover, fetal and placental weights were unchanged between the different treatment groups. The total number of proliferative cells also remained unchanged between the different treatment groups. However, the hypothesized subtype of cells thought to be affected with heparin treatment is proliferative trophoblast cells, which will be assessed using PHH3, cytokeratin 18 IHC double staining. Moreover, qRT-PCR analysis of certain trophoblast markers in the placental labyrinth zone will be carried out.

R12. THE USE OF microRNA TRANSGENESIS FOR THE REPROGRAMMING OF EMBRYONIC STEM CELLS TO TROPHOBLST STREM CELLS

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Early human placental development is poorly understood largely because of its inaccessibility. Consequently, the mechanisms responsible for many placental pathologies remain unknown, leaving diseases untreated, and making it imperative that we understand this early phenomenon. During mammalian embryonic development, the first differentiation event results in the emergence of the the trophectoderm, which will give rise to trophoblast stem cells and all of the trophoblast cell types that will comprise the placenta. Simultaneously, the inner cell mass is formed, which will go on to form the embryo proper. Trophoblast stem cells differentiate from this trophectoderm layer and will later differentiate again into syncytiotrophoblast and cytotrophoblast cells. While these cell fate decisions can be studied in the mouse model, access to human blastocysts for research is limited. Therefore a human cell based system would be an excellent solution to characterize both the early cell fates and trophoblast development. Derivation and analysis of human trophoblast stem cells (TSCs) could provide insight into these early origins. Reprogramming is a novel method used for cell transformations. We propose the use of microRNA-based reprogramming to generate human trophoblast stem cells from other cell types. Additionally, this method will be used to derive patient-specific TSCs in order to study the many kinds of placenta-related pathologies. MicroRNAs are recently discovered, small post transcriptional regulators that bind to mRNA leading to their inactivation or degradation and therefore inhibiting their translation. It is now hypothesized that miRNAs may target up to 60% of our genes, with over 1000 miRNAs encoded by the human genome. Research conducted by Dr. Brian Cox utilized a data mining technique, identifying microRNAs specific to TSCs, which inhibit translation of mRNA transcripts crucial to embryonic stem cell (ESC) biology. We hypothesized that transfecting ESCs with microRNA 322, 15b, and/or 467g would cause a switch to a trophoblast stem cell-like state. This hypothesis was first tested in mouse ESCs. Using a piggyback vector system with a doxycycline inducible promoter, stable embryonic stem cell lines were generated, with inducible expression of microRNA 322, 15b and 467g. In long term induction experiments, mir15b-transfected colonies from 2 cell lines were induced with doxycycline (10ng/ul) for 11 days. Colony morphology began resembling that of trophoblast stem cells at day 5. RNA was extracted from day 5, 7, 9, 11-cultured colonies, and cDNA was synthesized. qPCR analysis revealed a decreasing trend of ESC marker Pou5f1 and a stabilization of TSC marker Cdx2 in both cell lines. MicroRNAs play an important role in cell biology and their use in reprogramming may serve as a faster and more efficient alternative to more commonly used transcription factors. Future experiments will involve the introduction of a microRNA sponge (synthesized to target our microRNAs of interest) into the newly derived TSCs, which will block the action of the transfected microRNA and cause a regression back to an ESC. This will provide a further understanding of the mechanism of action of these microRNAs.

R13. PRENATAL GLUCOCORTICOID TREATMENT IMPAIRS TGF-β1 STIMULATION OF MULTIDRUG RESISTANCE IN FETAL BRAIN ENDOTHELIAL CELLS

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Pregnant women at risk of preterm birth are treated with synthetic glucocorticoids (sGC) to mature fetal lungs. This treatment is given in late gestation coincident with the most rapid period of fetal brain growth, primarily due to differentiation of astrocytes. Transforming growth factor-beta1 (TGF-β1) is released from differentiating astrocytes and increases activity of the blood-brain barrier (BBB) efflux transporter P-glycoprotein (P-gp; encoded by *abcb1*) in fetal and neonatal brain endothelial cells (BECs). P-gp is important for fetal brain protection against xenobiotics, including many therapeutic drugs taken by pregnant women. However, it is unknown if prenatal exposure to sGC, which affects brain development, also affects brain protection by P-gp. We hypothesized that prenatal exposure to sGC would alter the effects of TGF-β1 on P-gp actvity in fetal BECs. Pregnant guinea pigs were treated with dexamethasone or saline on gestational day (GD) 50 and euthanized on GD51. BECs were extracted from GD50 fetal brains and treated with TGF-β1 (1, 3.3, or 10 ng/ml) for 8 hours. P-gp activity and TGF-β1 receptor mRNA levels were assessed in BECs using fluorescent spectroscopy and qRT-PCR, respectively. We quantified SMAD7 mRNA levels to assess TGF-β1 cell sensitivity. TGF-β1 stimulation of P-gp was significantly attenuated in sGC-exposed BECs compared to control BECs. In addition, TGF-β1 increased *abcb1* ($p<0.001$) and SMAD7 mRNA ($p<0.01$) in control BECs with no effect on these levels in sGC-exposed BECs. Further analysis revealed that prenatal exposure to sGC resulted in up-regulation of TGF-β1 receptors *alk1* and *tgfbr2* mRNA expression compared to control ($p<0.05$). Prenatal sGC treatment impairs TGF-β1 stimulation of P-gp activity in fetal BECs and blunts responsiveness of BECs to TGF-β1. Interestingly, this decreased responsiveness is similar to that seen in normal postnatal guinea pig BECs suggesting that antenatal sGC exposure is capable of inducing BBB maturation.

R14. GENERATING PARTIALLY REPROGRAMMED KIDNEY TUBULE CELLS AND EVALUATING THEIR USE IN KIDNEY CELL THERAPY

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After acute tubular damage, the kidney has an intrinsic ability to repair itself, but in humans this repair process is often too slow. Moreover, this repair ability is very limited in patients with chronic renal disease. Other than kidney transplantation, no current treatments effectively alleviate kidney damage and improve the quality of life for patients. Hence, there is a need to develop new treatments that can accelerate the repair process and improve the quality of life for patients with renal diseases. Growing evidence suggests that tubular regeneration occur through the dedifferentiation, proliferation, migration and differentiation of surviving tubule cells to replenish cells in the denuded tubules. Given this, we hypothesize that an exogenous source of proliferative, partially reprogrammed (dedifferentiated) tubule cells will greatly improve the speed of tubule repair and the time to recovery. Partially reprogrammed cells (or reprogramming intermediates) are cells that have not completed the reprogramming process to become true pluripotent stem cells but should have acquired some pluripotent properties (i.e. proliferation) and retained some somatic properties. *In vitro* and *in vivo* methods will be used to evaluate the repair and regenerative potential of the tubule reprogramming intermediates. In our proof-of-principle experiment, we generated partially reprogrammed cells from unsorted Oct4-GFP mouse kidney cells using the doxycycline-inducible piggyBac transposon system containing the Yamanaka factors (c-Myc, Klf4, Oct4, Sox2). The reprogramming intermediates proliferate well and can easily form tubules in

culture. When these cells were reseeded into decellularized adult mouse kidney extracellular matrix scaffolds, they survived, repopulated tubular segments and expressed mature cell markers. Our preliminary data shows that reprogramming intermediates has great potential in repopulating denuded tubular segments and thus in kidney tubule repair. Next, we will focus on generating proximal tubule reprogramming intermediates (mouse and human) to repair proximal tubules, where most of the tubular damage occurs.

R15. IDENTIFYING AND QUANTIFYING EXOSOMES IN HUMAN SEMINAL FLUID

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Exosomes are membrane-bound microvesicles produced by cells and secreted into the surrounding extracellular matrix. They range in size from 30-150 nm and are secreted from nearly every tissue in the body. The recent discovery that these small vesicles contain functional proteins, mRNA, and miRNA suggest an important role in cell-to-cell communication. Seminal fluid, in particular, contains a mixture of microvesicles predominately originating from the prostate (prostasomes) and epididymis (epididymosomes). Our objective was to validate and develop an assay for the isolation and analysis of exosomes in human seminal fluid. Using a commercial extraction buffer (ExoQuick: System Biosciences) that selectively precipitates particles less than 1000 nm in size, we isolated nanoparticles from human seminal fluid and visualized them using NanoSight Tracking Analysis (NTA). NTA uses Brownian motion to measure the size of each particle detected and compiles a distribution curve based on concentration. The size distribution of nanoparticles was compared between fresh and frozen (-80 C) aliquots of each sample to validate the use of previously frozen samples. Preliminary NTA results indicate a shift towards larger sized nanoparticles in the frozen samples compared to fresh suggesting aggregation. Protein extracted from the isolated nanoparticles from the same fresh and frozen samples were run on an SDS gel. The profile shows numerous proteins ranging in size from approximately 15- 130 kDa. No difference was observed between the two treatment groups.

R16. LUNG TISSUE ENGINEERING: DECELLULARIZED LUNG SCAFFOLDS FOR EMBRYONIC STEM CELL DIFFERENTIATION

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The interaction of stem cells with the surrounding matrix environment is crucial for cell fate, as cell adherence, proliferation, spatial arrangement, and function are strongly influenced by matrix composition and structure. The development of biomatrices that recapitulate the *in vivo* environment is a key component to driving differentiation of pluripotent cells into lung endoderm precursors and ultimately transplantable, fully functional, lung tissues. Herein we demonstrate that decellularized lungs with intact matrix composition, devoid of all cellular components, can promote the differentiation of embryonic stem cells (ESC) into lung epithelial cells. Rat cadaveric lungs were decellularized by sequential tracheal lavages and retrograde pulmonary arterial perfusion using a range of physical, chemical, and enzymatic treatments. Histological staining, immunofluorescence, electron microscopy, and tensile testing confirm decellularization and preservation of matrix proteins such as collagen I/IV and laminin. Murine ESC ($Nkx2\text{-}1^{mCherry}$) were then seeded onto scaffolds following endoderm induction using activin. Seeded cells start to express the earliest known lung lineage marker Nkx2-1 and adopt an epithelial-like tubular organization. Cells further differentiate to epithelial cells found in the trachea and bronchioles (Clara cells, ciliated cells, basal cells), with continued culture on scaffolds. With the additional of fibroblast growth factors differentiation to a more distal cell population (alveolar regions) is observed. This work demonstrates that decellularized lung scaffolds effectively recapitulate the lung developmental milieu by supporting the adherence, proliferation, and differentiation of murine embryonic stem cells into mature populations of lung epithelia. Current work is focused on further maturation of cell-matrix constructs for transplantation purposes.

R17. EVALUATING CHANGES IN GENOMIC INTEGRITY AFTER OVERNIGHT INCUBATION OF HUMAN SPERMATOZOA

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Telomeres perform multiple roles essential to stabilization and protection of DNA, and are indicative of cellular replicative capacity. In spermatozoa, telomeres are highly elongated and hypothesized to support subsequent cellular divisions in early blastocyst development. Previous studies have demonstrated that telomeres are susceptible to reactive oxygen species (ROS) damage by which are abundant in sperm samples in vitro. IVF sperm samples are kept overnight in the event of unsuccessful fertilization for Rescue ICSI, exposing them to ROS. After incubation, sperm vitality and motility decline but the majority of the sample can still be used for Rescue ICSI. Sperm are selected based on motility and morphology, which provides no information on DNA quality. The purpose of the study was to determine if overnight incubation is detrimental to sperm telomeres and the DNA in which they protect. We hypothesized that exposure to ROS during overnight incubation would cause shortening of sperm telomeres and promote fragmentation of DNA. We evaluated changes in sperm parameters (motility and vitality), DNA integrity, and telomere length after overnight incubation to determine the effects of this process on sperm DNA. Sperm were prepared according to standard IVF protocols, with an important modification: incubation overnight at a 45° angle to create a gentle "swim up" effect. Three aliquots were analyzed: post-wash on the day of collection, and post-incubation from the upper and lower fractions. Each sample was subjected to Computer-assisted semen analysis (CASA), vitality staining, and DNA extraction for evaluation of DNA and telomere integrity. DNA quality was assessed using gel electrophoresis of undigested DNA, and telomere integrity assessed using terminal restriction fragment (TRF) analysis. This is a work-in-progress. At the time of submission, it was apparent that sperm isolated from each fraction of the incubation process possessed high quality DNA, despite noticeable decline in motility and viability after incubation. This finding was different from what we expected, and indicates that sperm DNA integrity is conserved despite decline in sperm activity and membrane integrity as indicated by vitality staining. Telomere integrity of each fraction will be assessed to determine if changes in telomere dynamics occurred after ROS exposure during incubation.

R18. PROBIOTIC *LACTOBACILLUS RHAMNOSUS* GR-1 SUPERNATANT BLOCKS LIPOPOLYSSACHARIDE (LPS)-INDUCED PRETERM BIRTH IN PREGNANT CD-1 MICE.

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Intrauterine infection/inflammation is involved in 25-40% of preterm births (PTB). Probiotics have been proposed to protect against PTB, supported by our previous studies showing that *Lactobacillus rhamnosus* GR-1 supernatant (GR-1 SN) reduces lipopolysaccharide (LPS)-induced pro-inflammatory cytokine TNFα output by human placenta, decidua and amnion cells *in vitro*. We hypothesized that GR-1 SN prevent LPS-induced PTB *in vivo*. Pregnant CD-1 mice were given 2 doses of 0.2mL GR-1 SN or saline intra-peritoneally

on gestational day (gd) 14 and 15. Intra-uterine infusion of LPS (125ug or 65ug) or saline was given 15-30 min after the last dose of GR-1 SN on gd 15. Mice (n=10/ group) were monitored until term (gd 20) for PTB, defined as delivery of the first pup within 48 hours post LPS infusion. A second set of mice was sacrificed 8 hrs after LPS (125ug) infusion for measurement of cytokines and chemokines in the maternal plasma and amniotic fluid by Bioplex (n=10/group). Statistical significance ($p<0.05$) was assessed using One-Way ANOVA or ANOVA on ranks followed by Newman-Keuls test. GR-1 SN decreased LPS-induced PTB rate from 94% (LPS 125ug) to 57% (LPS 125ug + GR-1 SN) and from 81% (LPS 65ug) to 53% (LPS 65ug + GR-1 SN). Mice not delivering preterm delivered live pups at term. LPS (125ug) infusion significantly increased the concentrations of IL-6, TNFα, MIP-1α and MIP-1β in amniotic fluid by 149-, 3-, 10-, and 39- fold respectively compared to saline, which were decreased significantly with GR-1 SN pretreatment to 32-, 2-, 3- and 14-fold respectively, compared to saline. In the maternal plasma, GR-1 SN pretreatment decreased significantly the LPS-induced increases in IL-6, TNFα and MIP-1β. We have shown for the first time *in vivo* that supernatant of probiotic *Lactobacillus rhamnosus* GR-1 attenuates LPS-induced PTB and associated elevations in inflammatory cytokine and chemokine.

R19. THE HORMONAL REGULATION OF THE NOVEL REPRODUCTIVE PEPTIDE PHOENIXIN IN NPY AND GNRH HYPOTHALAMIC MODELS BY ESTROGEN, INSULIN AND DEXAMETHASONE

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Phoenixin is a newly discovered reproductive peptide that is most significantly expressed in the hypothalamus. This novel peptide increases GnRH- stimulated basal LH secretion and up-regulates GnRH receptor mRNA in the anterior pituitary. However to date no studies have looked at the regulation of phoenixin at the level of the hypothalamus or at the roles it could play in energy homeostasis. As phoenixin is highly expressed in the hypothalamus, we have used immortalized hypothalamic cell lines developed by our laboratory to help elucidate the regulation of phoenixin in the hypothalamus by the reproductive hormone estrogen, the metabolic hormone, insulin and the glucocorticoid dexamethasone. Phoenixin expression has been detected by PCR in many of our hypothalamic cell lines and the mHypoA-NPY/GFP and mHypo-GnRH/GFP cell lines were selected to as models of central neurons in the metabolic and reproductive systems, respectively. Both lines express estrogen, insulin and glucocorticoid receptors. The mHypoA-NPY/GFP cell line was treated with 20 nM of dexamethasone and the relative phoenixin mRNA expression was determined by qRT- PCR over a 24-hour time course. Treatment of NPY/GFP hypothalamic cells with dexamethasone caused a significant increase in phoenixin mRNA expression after 4, 8 and 24 hours. This suggests that acute stress up-regulates phoenixin transcription in the hypothalamus. There were no significant changes in phoenixin mRNA with treatment of 10 nM 17- β estradiol or with 10 nM insulin on either cell line. The molecular pathways involved in regulating phoenixin by these hormones will also be determined by Western blot experiments. Current experiments are underway to investigate the effects of phoenixin treatment in both hypothalamic cell lines. These experiments will help us to understand the role that phoenixin may play in reproduction and energy homeostasis at the level of the hypothalamus and expand our knowledge of the neuroendocrine axis. These studies may also implicate phoenixin in reproductive or metabolic disorders.

R20. THE ROLE OF TGFβ IN REGULATING ACID CERAMIDASE EXPRESSION IN THE HUMAN PLACENTA

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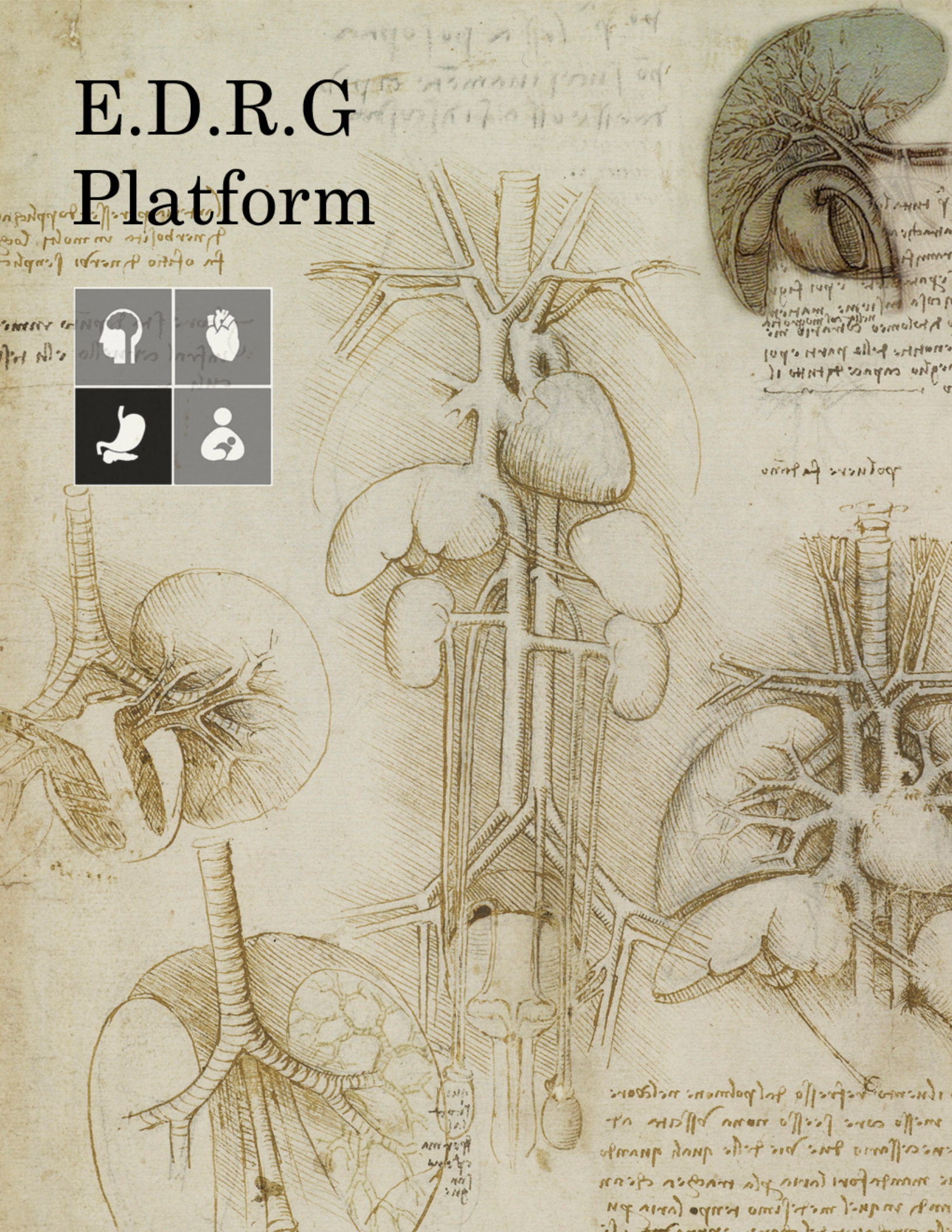
Sphingolipids are active mediators of cell proliferation and apoptosis. Central to sphingolipid metabolism is ceramide (CER), which is known to promote cell death. CER levels are maintained through its synthesis and breakdown, via the action of enzymes. In particular, acid ceramidase (AC) is responsible for hydrolyzing CER into sphingosine, which in turn is processed into sphingosine 1 phosphate (S1P) by sphingosine kinases (SphK1/2). We have previously established the importance of altered TGFβ3 levels in preeclampsia and intrauterine growth restricted pregnancies (IUGR); as well as we have recently found altered sphingolipid profiles in preeclamptic placentae. However, no information is available on the role of TGFβs in regulating sphingolipid metabolism in the human placenta. We hypothesize that TGFβs, particularly TGFβ3, regulate AC expression in trophoblast cells thereby altering the sphingolipid rheostat and impacting cell fate. Studies were conducted using human choriocarcinoma JEG3 cells cultured under standard conditions, treated with TGFβ1, TGFβ3 (5 ng/ml), or control vehicle (DMSO), and collected at 3, 8, and 24 hours. AC mRNA levels were evaluated by quantitative-PCR analysis, AC protein expression was examined by western blotting, and its sub-cellular distribution was assessed by immunofluorescence analysis. We found that TGFβ3, and to a lesser extent TGFβ1, caused a significant increase in AC mRNA expression in JEG3 cells following 3 and 8 hours of treatment; while AC protein levels increased 24 hours post-treatment. TGFβ3 also caused AC to redistribute to the cell boundaries where it colocalized with ZO1, a marker of tight junctions. Upon silencing of Smad2, AC expression decreased and was less apparent at the cell boundaries indicating a Smad2-dependent regulation. Our data implicate a novel role for TGFβ3 in regulating AC expression and cellular distribution in JEG3 cells, thereby supporting a role for this growth factor in modulating sphingolipid metabolism thus contributing to trophoblast cell fate events.

R21. DECIDUAL NEUTROPHILS: A NOVEL ROLE IN SECOND TRIMESTER VASCULAR REMODELING

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During early pregnancy, trophoblast-mediated vascular remodeling transforms spiral arteries into large, dilated, low-resistance vessels capable of providing increased blood flow to the intervillous space. Absent in first trimester decidua, neutrophils are not believed to contribute to vascular remodeling; however, we detected a large 2nd trimester (TM) neutrophil population. To investigate a potential role for neutrophils, 2nd TM leukocytes were isolated from decidual tissue and peripheral blood for analysis and identification by flow cytometry of leukocytes, neutrophils, and selected chemokine receptors (CD45, CD15, CD66b, CD181, CD182, CD183, CD184). Immunohistochemistry was performed against CK, HLA-G, CD66b and neutrophil elastase to evaluate decidual distribution of neutrophils. Neutrophil distribution was also examined in implantation sites of CD-1 mice at gestational days 6, 8, 10, 12, 14, and 18 using an F7/4 antibody. Immunofluorescence was conducted on 2nd TM decidua to investigate co-localization of neutrophils and angiogenic factors. Human uterine microvascular endothelial cells (hUtMVEC) were cultured with neutrophils in serum-free or 2nd TM decidua-conditioned media (DCM) in angiogenesis assays. Decidual neutrophils (DN) and peripheral blood neutrophils (PBN) demonstrate a significantly different chemokine receptor profile. Neutrophils were detected in 2nd TM decidua distributed throughout tissue and concentrated around blood vessels, while murine neutrophils are significantly greater at days 10 and 12. Only DN co-localize with angiogenic factors VEGF, Arg-1, and CCL-2 in decidua. DCM treatment of PBN also drives expression of these angiogenic factors and promotes in-vitro angiogenesis, as hUtMVEC show more advanced tube formation in culture with DCM-treated PBN compared to control SFM neutrophils. The presence of decidual neutrophils together with the production of angiogenic factors suggests a role for neutrophils in 2nd TM uterovascular remodeling. Further investigation will determine more specific mechanisms of decidual neutrophil functions.

E.D.R.G Platform



ATF6β DEPLETION INCREASES SUSCEPTIBILITY OF PANCREATIC β-CELLS TO ENDOPLASMIC RETICULUM STRESS-INDUCED APOPTOSIS

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Endoplasmic reticulum (ER) stress has been implicated as a causative factor in the development of pancreatic β-cell dysfunction and death resulting in type 2 diabetes. Activating transcription factor 6 (ATF6) is a critical component of the Unfolded Protein Response in cells undergoing ER stress. As unfolded and misfolded proteins accumulate in the ER, ATF6 is activated and contributes to the induction of various ER chaperones and components of the ER-associated degradation pathway, which assist in maintaining ER homeostasis in the cell. We have previously demonstrated that the ATF6α isoform is required for pancreatic β-cell survival under basal and ER stress conditions. However, no studies to date have investigated the role of the ATF6β isoform in pancreatic β-cells. Using an ATF6β-specific antibody we detected expression of full-length ATF6β in various insulinoma cell lines and rodent islets and the induction of the active form (ATF6βp60) under ER stress conditions. However, knockdown of ATF6β in INS-1 832/13 insulinoma cells did not affect mRNA or protein induction of known ER stress response genes (Grp78, Grp94, Herp, Hrd1) in response to tunicamcin-induced ER stress. Thus, ATF6β does not appear to be required for the induction of genes known to be induced by ATF6α, although insulinoma cells deficient in ATF6β had a compensatory increase in ATF6α levels. Interestingly, knockdown of ATF6β increased the susceptibility of β-cells to ER stress-induced apoptosis, as monitored by the levels of pro-apoptotic markers (cleaved caspase 3, PARP, CHOP). Together, our results suggest that ATF6β is not required for induction of major ER stress response genes, but is required to maintain cell survival in β-cells undergoing ER stress. Thus, ATF6β has a pro-survival role as part of the ER stress response, although the exact complement of genes regulated by this ER stress response sensor is unknown and the subject of current investigation.

miR-142-3p DIRECTLY REGULATES AUTOPHAGY-DEPENDENT GENE ATG16L1 IN CROHN'S DISEASE

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Numerous genome-wide association studies demonstrate that a variant in the autophagy-dependent gene ATG16L1 is associated with Crohn's Disease (CD), implicating autophagy in the development of inflammation. The CD variant may affect autophagy in a unique pathway, possibly through the RNA-induced silencing complex (RISC) that uses a strand of miRNA as a template for recognizing complementary RNA. Emerging data show that altered regulation of pro and anti-inflammatory genes by miRNA is involved in many disease states including inflammatory bowel disease. miRNA are short non-coding RNA that bind to the complementary 3' untranslated region (UTR) of the target mRNA to repress translation and promote degradation. We hypothesize that the miRNA silencing pathway may contribute to the pathogenesis of CD through regulation of ATG16L1. Bioinformatic target prediction tools such as miRecord were employed to identify miRNA predicted to target ATG16L1 3'UTR. Out of 36 identified putative miRNA, miR-142-3p was selected for further characterization based on homology across species and its reported role in inflammatory states. HeLa cells were transfected with varying concentrations of a miR-142-3p mimic or inhibitor and quantitative real time PCR for ATG16L1 performed. To assess the impact on autophagy, cells were co-transfected with LC3-GFP in the presence or absence of rapamycin and imaged by confocal microscopy to quantitate autophagic puncta. In comparison to control cells, a decrease in ATG16L1 transcripts was detected in cells treated with the miR-142-3p mimic. Consistently, a reduction in LC3 puncta reflecting a decrease in autophagosome abundance was detected in cells transfected with miR-142-3p mimic, when compared to sham-transfected cells. In contrast, an increase in LC3 puncta was detected in cells treated with the inhibitor. Taken together, these results indicate that miR-142-3p can directly regulate ATG16-L1 and repress autophagy. We propose that miRNA regulation of the autophagy pathway could be critical in the pathogenesis of CD.

EFFECTS OF TIMING OF SATURATED FAT AND LIQUID SUGAR INTAKE ON OBESITY IN RATS AND CIRCADIAN RHYTHMS IN HYPOTHALAMIC CELLS.

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Overeating is associated with obesity. However, not only the amount, but also the timing of caloric intake could be contributing to metabolic disturbances. The mechanism via which this happens is not well understood. In a rat model we assessed which nutrient attributes most to this time-of-day-dependent weight gain. 48 male Wistar rats were subjected to chow *ad libitum* or a choice diet with saturated fat, a 30% sugar solution, chow and tap water; either *ad libitum* ('ad lib') or with daytime restricted access to fat ('LF') or sugar ('LS'). Food intake and body weight gain were studied during five weeks. Energy expenditure was studied in the third week in calorimetric cages. All diet groups showed hyperphagia compared to the control group. Rats restricted to daytime sucrose (LS) gained most body weight per ingested calorie and showed a lower and inversed respiratory exchange ratio (RER) compared to the other groups, despite equal caloric intake, locomotor activity and heat production. Thus, timing of sugar intake seems important for body weight gain, possibly due to a change in energy balance. In order to understand the effects of high fat and sugar at the cellular level, the immortalized, hypothalamic mouse cell line mHypoE-37 was used, which exhibits robust circadian rhythms of core clock genes. We found that incubation with 25 μM palmitate or 5.5 mM glucose alters the period and amplitude of Bmal1 and Per2 mRNA, indicating that fatty acids and glucose could disrupt normal clock gene rhythmicity in hypothalamic neurons, possibly leading to metabolic disruptions. In future experiments we will expose this cell model to different temporal and nutrient paradigms to determine if the molecular clock is disrupted in these scenarios.

E.D.R.G.: Posters

E1. ROLE OF THE INTESTINAL EPITHELIAL INSULIN-LIKE GROWTH FACTOR-1 RECEPTOR IN GLUCAGON-LIKE PEPTIDE-2-MEDIATED ENHANCEMENT OF INTESTINAL BARRIER FUNCTION

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Glucagon-like peptide-2 (GLP-2) is a potent nutrient-dependent intestinal growth factor for the treatment of Short Bowel Syndrome. This intestinotrophic hormone enhances mucosal growth and intestinal barrier function. We have previously shown that the proliferative effects of GLP-2 are mediated through the actions of another growth factor, insulin-like growth factor-1 (IGF-1), on its receptor in the intestinal epithelium (ieIGF-1R). The aim of this study is to investigate the barrier effects of GLP-2 and the mechanisms by which GLP-2 modulates intestinal permeability using the inducible ieIGF-1R knockout (KO) mouse model. KO mice and littermate IGF-$1R^{fl/fl}$ control mice were treated with either GLP-2 or vehicle for 10 days. In vivo, gastrointestinal permeability was measured by paracellular leak of a relatively impermeant marker, fluorescein-isothiocyanate dextran 4000. GLP-2 reduced permeability in control mice at 1.5h ($p<0.05$), but this response was abrogated in KO mice. In addition, Ussing chambers were used to assess barrier function of the jejunum, where the GLP-2 receptor is highly expressed. Jejunal resistance was increased ($p<0.001$) in control animals treated with GLP-2, as compared to vehicle controls. This GLP-2-mediated elevation in resistance was abolished in KO animals. The underlying tight junctional proteins were examined by immunoblotting and immunofluorescence. Jejunal expression of ZO-1 and occludin were not different between control and KO animals, with or without GLP-2 administration. However, there were changes in the subcellular distribution of these two barrier proteins. On the other hand, the tightening junctional protein claudin-7 was upregulated in control mice treated with GLP-2, consistent with more intense immunofluorescence. Claudin-7 upregulation by GLP-2 was absent in KO mice. Collectively, GLP-2-mediated enhancement of barrier function was reduced in the absence of the ieIGF-1R, and this can be attributed to disrupted subcellular localization and expression of the tight junctional proteins.

E2. INSULIN ANALOGUES GLARGINE AND DETEMIR DO NOT PROMOTE CHEMICALLY-INDUCED CARCINOGENESIS IN RATS

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Solid epidemiological studies support the link between insulin treatment of patients with diabetes and increased cancer risk. While more favorable insulin analogs have been developed to reduce the risk of hypoglycemia, their role as cancer promoters remains controversial according to epidemiological studies. Therefore, preclinical models are needed. We have used an established dual carcinogen rat model (methylnitrosourea(MNU) and azoxymethane(AOM)) of mammary and colorectal cancer to investigate the cancer promoting effect of long-acting insulin analogs. Female Sprague-Dawley rats were given one MNU injection(50mg/kg) and two AOM injections(15mg/kg) consecutively by weekly basis. All the rats were randomly assigned to four groups that were injected with vehicle(saline), NPH (prolonged formulation of unmodified insulin), or long-acting insulin analogs glargine or detemir. The groups were given their corresponding treatment for 6 weeks. Expectedly, the blood glucose levels decreased significantly(about 3mmol/l reduction) in all insulin treated groups compared to the control when measured 4-5h post-injection(peak insulin action). Also, glycosylated hemoglobin levels(A1c) significantly decreased in insulin treated groups. Rats treated with NPH(n=20) tended to have higher mammary tumor incidence (80% vs 61%), multiplicity(number of tumors per animal) and volume(mm^3) than controls (n=18) . Insulin glargine (n=19) or detemir group (n=22) did not show any difference in tumor incidence (53% with glargine and 73% with detemir), multiplicity, or volume compared to control(In fact, the glargine group showed less mammary tumor incidence(53%) compared to NPH group. There was no effect of any insulin on the total number of aberrant crypt foci(ACF; precursor of colorectal tumor) or crypt multiplicity compared to the control. In summary, NPH showed a tendency to promote only breast cancer but not colorectal cancer precursors. Consistent with ORIGIN study, neither insulin glargine nor detemir promoted cancer development.

E3. ROLE OF VESICLE-ASSOCIATED MEMBRANE PROTEIN 2 IN EXOCYTOSIS OF GLUCAGON-LIKE PEPTIDE-1

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Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted by endocrine L-cells of the distal gut in response to nutrients (e.g. Oleic acid (OA)) and hormones (e.g. Glucose-dependent Insulinotropic Peptide (GIP)).The molecular mechanisms underlying GLP-1 exocytosis are unknown although, as in other endocrine cells, granule fusion is likely mediated by the SNARE complex, which consists of SNAP-25 and Syntaxin on the plasma membrane, and vesicle-associated membrane protein (VAMP) on the granule. We thus hypothesized that VAMP2 plays an integral role in GLP-1 exocytosis. The presence of VAMP2 in GLUTag cells, a murine L-cell model, was demonstrated by RT-PCR and immunoblot. Transfection of the cells with pcDNA3-tetanus toxin light chain (TetX-LC) led to 55% cleavage of VAMP2 ($n=6$, $p<0.01$), and caused redistribution of co-transfected VAMP2-GFP, normally targeted to cytoplasmic and cell membrane compartments, to a peri-nuclear region in 98% of transfected cells (n=1307), consistent with TetX-LC cleavage of VAMP2. OA (1000 μM) and GIP (100 nM) increased GLP-1 secretion by 2.2- to 2.3-fold in pcDNA3-transfected GLUTag cells ($n=6$-8; $p<0.05$), responses that were completely abrogated in TetX-LC- transfected cells. Finally, total internal reflection fluorescence microscopy was used to examine GLP-1 granule exocytosis directly, at the single cell level. GLUTag cells co-transfected with the granule content marker, NPY-mCherry, and VAMP2-GFP demonstrated co-localization of the signals in 96% of the cells (n=1321). GLUTag cells were then transfected with NPY-pHluorin, which fluoresces upon exposure of exocytosing granules to the alkaline cell exterior. Preliminary data showed that 50mM KCl increased exocytosis by 1.79 fold (n=3) in control transfected cells, whereas cells transfected with pcDNA3-TetX-LC exhibited an 80% reduction in basal secretion, as well as a 62% decrease in stimulated secretion (n=5). Together, these studies suggest that VAMP2 plays an integral role in GLP-1 exocytosis.

E4. DUODENAL FATTY ACIDS WITH VARYING DEGREES OF SATURATION REGULATE GLUCOSE PRODUCTION VIA CCK-DEPENDENT AND INDEPENDENT MECHANISMS

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Diabetes and obesity are characterized by elevated glucose levels due partly to an elevation of hepatic glucose production (GP). However, the mechanisms underlying the regulation of GP in healthy and obese/diabetic settings remain to be elucidated. Duodenal lipid metabolism is demonstrated to trigger a gut-brain-liver neuronal axis to lower GP in rats in vivo. More specifically, infusion of the fat emulsion Intralipid into the duodenum increases long chain fatty acids (LCFA) levels. LCFAs are then converted to LCFA-CoA to trigger a PKC-δ → CCK (cholecystokinin) → CCK1 receptor → PKA signaling cascade to trigger a neuronal network to lower hepatic GP. Intralipid is a source of essential fatty acids consisting of both saturated

(SFA) (containing no double bonds) and mono- and polyunsaturated fatty acids (MUFA, PUFA) (containing ≥1 double bond(s)). More specifically, Intralipid contains the highest percentage of linoleic acid (PUFA) and oleic acid (MUFA). Whether these individual fatty acids within this emulsion lower GP and utilize this same signaling cascade in the duodenum currently remains to be explored. Therefore, we investigated whether linoleic acid and oleic acid lower GP when infused into the duodenum and whether they both utilize similar mechanisms in normal rats in vivo. First, intraduodenal infusion of oleic acid or linoleic acid lowered GP during the pancreatic (basal insulin) euglycemic clamps. Second, co-infusion of oleic acid with the CCK1 receptor inhibitor, MK-329, abolished the ability of oleic acid to lower GP. Interestingly, co-infusion of linoleic acid with MK-329 did not block the ability of linoleic acid to lower GP. In summary, these data together illustrate that fatty acid sensing in the duodenum can lower glucose production via CCK-dependent and independent mechanisms.

E5. THE EFFECT OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-4 ON GLUCAGON LIKE PEPTIDE-2 BIOACTIVITY ON MURINE INTESTINE

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Glucagon-like peptide-2 (GLP-2) is a peptide hormone, released from the intestinal L cells, known to play an important role in stimulating the growth and function of the gut. Degredation resistant analog of GLP-2 have recently approved for use for patients with short bowel syndrome. Binding of GLP-2 to the GLP-2 receptor on intestinal subepithelial myofibroblasts (ISEMF) that underlie the epithelium activates a signalling mechanism that promotes intestinal epithelial cell (IEC) growth, as well as inducing a number of other beneficial effects that improve gut function. However, the exact signalling mechanism through which GLP-2 exerts its beneficial effects is not known. Previous work has established that both IGF-1 and the IGF-1 receptor on the IEC are essential for GLP-2-enhanced IEC proliferation. Furthermore, preliminary data suggest that, in addition to IGF-1, ISEMFs express insulin-like growth factor binding protein 4 (IGFBP-4) which is a regulatory protein that can enhance or inhibit IGF-1 action. Thus, we hypothesize that IGF-1 signalling between ISEMFs and the IECs in response to GLP-2 is modulated by co-release of IGFBP-4. Primary ISEMF cell lines were isolated from wild type mice for this study. Through immunocytochemistry these ISEMFs were found to be positive for alpha smooth muscle actin, and vimentin, and negative for desmin, consistent with previously published characterization of ISEMFs. To study the changes in relative expression levels of IGFBP-4 in response to GLP-2, ISEMFs were treated with GLP-2 (10^{-8}M) and mRNA was isolated at 30 min, 2, 4, 8, 12, and 24 hours after treatment. Relative IGFBP-4 mRNA levels, assessed using quantitative real-time PCR, suggest a trend of increased expression at 4 hours and 12 hours after GLP-2 treatment. The results suggest that the IGF-1-modulating protein, IGFBP-4, may have a role in the GLP-2 enhanced IEC growth within the established IGF-1 – IEC-IGF-1 receptor pathway.

E6. IDENTIFICATION OF GABA A RECEPTOR BETA3 (GABRB3)-INTERACTING PROTEINS AND NOVEL FUNCTIONS OF GABRB3 IN PANCREATIC BETA-CELLS

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The A type of gamma-aminobutyric acid receptor (GABAAR), an important route for GABA signaling, is a pentameric ligand-gated ion channel that is composed of two alphas, two betas and a third type of subunit. Among these subunits, the beta3 subunit (GABRB3) is present in a large proportion of GABAAR in the pancreatic beta-cells and is depleted in diabetic condition, implying its importance in the regulation of beta-cell functions. We have found that expression and nuclear localization of GABRB3 are significantly increased during beta-cell mitosis. To investigate the specific role of GABRB3 in modulating beta-cell functions, we performed immunoprecipitation using anti-GABRB3 antibody in the beta-cell model INS1 cells. Mass spectrometry analysis revealed that GABRB3 interacts with nuclear transport regulators (karyopherin alpha6), transcriptional regulators (poly-U binding splicing factor 60) and other nuclear proteins, suggesting that GABRB3 translocates to the nucleus and exerts certain functions in the nucleus. Karyopherin alpha6 is an importin protein that binds with nuclear localization signal (NLS)-containing proteins to mediate nuclear import. We have identified an NLS domain on GABRB3 within the intracellular loop between transmembrane (TM) 3 and TM4 domain. Indeed, INS1 cells expressing NLS domain-mutated GABRB3 showed remarkable impairment of GABRB3 nuclear localization, suggesting that the NLS domain is required for GABRB3 nuclear translocation, likely by interacting with karyopherin alpha6. GABRB3 may subsequently affect transcriptional activation directly or indirectly by binding with other nuclear proteins. Our findings suggest that GABRB3 exerts a dual function in pancreatic beta-cells by conveying extracellular GABA signal at the plasma membrane and by mediating transcriptional processes in the nucleus of beta-cells.

E7. LIPID SENSING IN THE ILEUM REGULATES GLUCOSE PRODUCTION

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Recent studies demonstrate that duodenal lipid sensing triggers a neuronal network to inhibit glucose production (GP). Although it is believed that nutrient absorption occurs mainly in the duodenum, studies have reported that in normal rodents and dogs, ingested nutrients reach the distal gut. An injection of labeled-Intralipid increases radioactivity in the duodenum, jejunum and ileum shortly after 15-30 min in rodents, while a small amount of nutrients reach the ileum after a normal meal as well in humans. In fact, intra-ileal infusion of lipids lowers food intake in rodents and humans. These findings collectively raise the possibility that lipid-sensing mechanisms in the ileum are activated shortly after a meal, and such activation is crucial to maintain glucose homeostasis. We here first inserted a catheter into the lumen side of the ileum 14 cm proximal to the cecum of a normal male Sprague-Dawley rats (280-300 g), followed by intravenous catheterization where the internal jugular vein and carotid artery were catheterized for infusion and sampling. The pancreatic (basal insulin) euglycemic clamp was performed in combination with glucose tracer dilution methodology to assess changes in glucose kinetics. During an intra-ileal saline infusion (50 min), the glucose infusion rate (GIR) required to maintain euglycemia was 0.9 ± 0.6 mg/kg/min. In contrast, intra-ileal infusion of Intralipid increased the GIR to 5.2 ± 2.2 mg/kg/min ($p < 0.05$). This was due to an inhibition of GP from 9.9 ± 1.6 (saline) to 4.8 ± 0.9 mg/kg/min ($p < 0.01$) rather than changes in glucose uptake (10.8 ± 1.6 saline vs. 10.0 ± 1.7 Intralipid). Thus, we here demonstrate, for the first time to our knowledge, that lipid sensing in the ileum lowers GP. Further experiments will aim to dissect the underlying signaling mechanisms of lipid-sensing mechanisms in the ileum *in vivo*.

E8. CENTRAL ADMINISTRATION OF GLUCAGON ACTIVATES GLUCAGON RECEPTORS TO INHIBIT GLUCOSE PRODUCTION IN MICE IN VIVO

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The observation that diabetes and obesity are associated with defects in hypothalamic hormone signaling cascades highlights the importance of central hormone regulation of glucose production (GP) and glucose homeostasis in normal and disease states. Previously, our lab revealed a novel gluco-regulatory role of glucagon in the hypothalamus of rats. Direct administration of glucagon into the mediobasal hypothalamus of rats under pancreatic (basal-insulin) euglycemic clamp conditions surprisingly lowered

GP, which is in contrast to the hormone's systemic effect on glucose homeostasis. Whether these inhibitory metabolic effects of central glucagon are present in normal and glucagon receptor knockout (Gcgr-/-) mice currently remain to be investigated. Therefore, we performed central glucagon administrations first in C57BL/6 normal mice (n=9) during a pancreatic (basal insulin)-euglycemic clamp. Central infusion of glucagon, but not saline, increased the glucose infusion rate required to maintain euglycemia (saline= 11.1 ± 1.3 mg/kg/min vs. glucagon= 24.6 ± 2.3 mg/kg/min, $p<0.001$), and this was accounted for by a suppression in GP (saline= 19.8 ± 1.3 mg/kg/min vs. glucagon= 6.0 ± 0.9 mg/kg/min, $p<0.001$) as opposed to a difference in glucose uptake (saline= 30.2 ± 1.3 mg/kg/min vs. glucagon= 30.5 ± 1.9 mg/kg/min). In order to directly evaluate the role of central glucagon receptors in mediating this effect, we next performed the clamp experiments in Gcgr-/- (n=6) and their littermate controls, Gcgr+/+ (n=5). Central infusion of glucagon in Gcgr-/- mice failed to increase the glucose infusion rate (Gcgr-/-= 10.4 ± 1.6 mg/kg/min vs. Gcgr+/+= 27.3 ± 0.6 mg/kg/min, $p<0.005$) and lower GP (Gcgr-/-= 22.6 ± 1.8 mg/kg/min vs. Gcgr+/+= 5.7 ± 0.7 mg/kg/min, $p<0.005$) as compared to the Gcgr+/+ control mice. Taken together, these data illustrate that glucagon activates the central glucagon receptors to lower glucose production in mice in vivo.

E9. OLEATE-INDUCED β-CELL DYSFUNCTION IS MEDIATED THROUGH 'β-CELL INSULIN RESISTANCE'

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Chronic elevation of free fatty acids (FFA) plays a role during the development of β-cell failure in predisposed individuals. 'Lipotoxicity' refers to the adverse effects of FFA on β-cell function and mass. Using in vivo models of β-cell dysfunction, our lab has demonstrated that oxidative stress plays a causal role in lipotoxicity. Oxidative stress is known to phosphorylate serine/threonine residues of insulin receptor substrates (IRS), which impairs insulin-like growth factor-1 (IGF-1)/insulin signaling. Preliminary data using bisperoxovanadate (tyrosine phosphatase inhibitor), which prevented -cell lipotoxicity, suggest that impaired tyrosine phosphorylation is also involved in β-cell lipotoxicity. Thus, perhaps 'β-cell insulin resistance' is involved in β-cell lipotoxicity. To determine whether 'β-cell insulin resistance' plays a causal role in fat-induced β cell dysfunction we used mice with β-cell specific deletion of PTEN (negative regulator of insulin signaling). Mice with homozygous or heterozygous deletion of PTEN and littermate controls were intravenously infused with saline or oleate (0.3μmol/min) for 48h, followed by assessment of β-cell function in vivo using a one-step hyperglycaemic clamp (22mM). 48h fat infusion impaired β-cell function (assessed by the disposition index; DI) compared to saline infusion in control mice. Both homozygous and heterozygous β-cell specific PTEN-null mice were protected from the oleate-mediated decrease in DI. These data suggest that 'β-cell insulin resistance' contributes to FFA-mediated β-cell dysfunction and that partial upregulation of insulin signaling is sufficient to protect against FFA-mediated β-cell dysfunction.

E10. PROTECTIVE EFFECTS OF ANTI-INFLAMMATORY COMPOUNDS IN A HYPOTHALAMIC CELL MODEL WITH PALMITATE- AND TNFA-INDUCED INFLAMMATION

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Hypothalamic neurons and their innate immune responses to nutritional excess contribute significantly to the initial pathogenic changes in energy homeostasis that terminate in diet-induced obesity and type 2 diabetes mellitus. Development of effective and targeted strategies to combat these diseases requires detailed characterization of the initial hypothalamic events that are difficult to decipher *in vivo*. Here, we have generated a hypothalamic neuronal model immortalized from the embryonic rat (rHypoE-7) to investigate hypothalamic inflammation at the molecular level. Of significance, the rHypoE-7 model expresses essential inflammatory machinery and maintains a fully functional IKK-β/Nf-κB cascade that is activated by TNF-α and palmitate, as shown by phospho-specific antibodies. Using quantitative RT-PCR (qPCR), Western blotting, and ELISA, we have demonstrated inducible expression and secretion of key proinflammatory cytokines elevated in metabolic diseases, such as interleukin (IL) 1-beta, TNF-α, and IL-6. Pre-exposure of rHypoE-7 cells with omega-3 fatty acids, sodium salicylate, the IKK-beta inhibitor PS1145, and AICAR significantly dampened or inhibited the inflammatory response as assessed by qPCR. Given that these pro-inflammatory molecules induced insulin resistance in rHypoE-7 cells, as evident by a reduction in insulin-dependent AKT phosphorylation, future work will determine the ability of these anti-inflammatory reagents in restoring insulin signalling and ultimately the regulated expression of neuropeptides involved in energy homeostasis. This work was generously supported by BBDC and CIHR fellowships (LW) and CIHR, CRC, and CFI grants (DDB).

E11. NEURONAL CELL MODELS GENERATED FROM FAC-SORTED, IMMORTALIZED MIP-GFP MOUSE HYPOTHALAMIC PRIMARY CULTURES

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Generation of the mouse insulin promoter (MIP)-GFP mice allow researchers to easily obtain homogenous cultures of pancreatic β-cells and their identification in real time. Additionally, the MIP is thought to be inactive within the brain of transgenic animals, and thus confers β-cell specificity. However, given the limited resolution of IHC imaging and cellular heterogeneity, the possibility of active MIP within the hypothalamus remains. Since this brain region contributes to metabolic control, it must be determined if any neurons have MIP expression. We hypothesized that there may be a small subpopulation of neurons with active MIP. Thus we aimed to create an appropriate cell model to characterize these neurons. Immortalized cells were created from dispersed adult-derived hypothalamic tissue with a retroviral T-antigen construct. The immortalized cells were then FAC-sorted to isolate cells with GFP expression, thus providing a mixed culture of MIP-GFP neurons to characterize. GFP expression in MIP-GFP neurons was confirmed by ICC imaging, but preliminary results show that they neither produce nor transcribe insulin. However, robust insulin receptor mRNA expression was detected along with neuropeptide mRNA, such as neuropeptide Y, agouti-related peptide and vasoactive intestinal peptide. Current work is in progress to determine whether expression and secretion levels of these neuropeptides change as a result of varying levels of insulin using real-time quantitative PCR and secretion assay kits. Additionally, IHC co-staining of neuropeptides and GFP is in progress in MIP-GFP mouse brains slices to determine the *in vivo* localization of these neurons. Given the successful creation of MIP-GFP neuronal models, we have determined that MIP activity is not limited to β-cells. Therefore, future work using the MIP promoter as a β-cell specific marker must take into account of its activity within the hypothalamus of the transgenic animals.

E12. GABA PROMOTES β-CELL PROLIFERATION AND SURVIVAL VIA MECHANISM INVOLVING ACTIVATION OF AKT, ERK, AND CREB

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Gamma-aminobutyric acid (GABA), an important amino acid, is produced by pancreatic β-cells and plays a role in regulating insulin and glucagon secretion and islet β-cell function. We showed recently that GABA exerts regenerative and protective effect on the islet β-cells under both in vitro and in vivo conditions. Here, we illustrated the signaling pathways conveying the trophic effects of GABA in the islet β-cells. Western blot analysis showed that GABA time- and dose-dependently induced Akt, ERK, and CREB phosphorylation in clonal insulin-secreting INS-1 cells, which are key

mediators for cell proliferation and survival. Thymidine incorporation assay revealed that GABA induced cell proliferation in INS-1 cells, which was inhibited by pharmacological inhibition using PI3K inhibitor LY294002, MAPK inhibitor PD98059, and PKA inhibitor H89, suggesting the role of these pathways in mediating GABA-induced β-cell proliferation. Western blot and flow cytometry analysis showed that inflammatory cytokines i.e. a mixture of TNF-α (50 ng/ml), IFN-γ (50 ng/ml), and IL1-β (10 ng/ml) markedly induced apoptosis in INS-1 cells and isolated adult human islet β-cells, which was attenuated by GABA treatment. GABA also inhibited reactive oxygen species (ROS) production in INS-1 cells and human islets, suggesting that GABA protected β-cells from apoptosis by suppressing ROS levels. In isolated normal human islets, GABA significantly induced Akt phosphorylation, which however, was not observed in type 2 diabetic islets (T2D). Furthermore, GABA-induced ERK and CREB phosphorylation were exaggerated in T2D islets, altogether suggesting an altered GABA signaling pathway in T2D. In summary, our results suggest that GABA exerts trophic effects by promoting β-cell proliferation via PI3K/Akt, MAPK/ERK, and PKA/CREB signaling pathways, which are altered in T2D, and that GABA promotes β-cell survival via inhibition of apoptosis, partially through inhibiting ROS production.

E13. THE ROLE OF P21-ACTIVATED PROTEIN KINASE 1 (PAK1) IN HEPATIC GLUCOSE METABOLISM AND HOMEOSTASIS

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Pak1 is importantly involved in many cellular processes, including insulin secretion and muscle glucose uptake. We reported previously that Pak1 is a positive regulator of proglucagon (*gcg*) gene expression, and that *Pak1*$^{-/-}$ mice have reduced GLP-1 levels and impaired glucose disposal. The *Pak1*$^{-/-}$ mice were also found to carry a moderate defect in intraperitoneal pyruvate tolerance test (PTT), indicating a role of Pak1 in hepatic glucose production (GP). Here, we show that the defect in PTT became more severe when the *Pak1*$^{-/-}$ mice reached 12 months old, implicating Pak1 as a repressor of hepatic GP. These aged *Pak1*$^{-/-}$ mice have impaired insulin and GLP-1 responses following glucose challenge, whereas glucagon level and glucagon tolerance was comparable with wild-type controls. Unexpectedly, mouse primary hepatocytes treated with the chemical group I Pak inhibitor IPA3 showed dose-dependent attenuation of forskolin- and glucagon-stimulated GP. Examination of gluconeogenic gene expression showed that the aged *Pak1*$^{-/-}$ mice have elevated expression of *pepck* and *g6p*, two rate-limiting enzymes for gluconeogenesis. However, mouse primary hepatocytes treated with IPA3 showed attenuation of forskolin-stimulated *pepck* and *g6p* expression. As Pak1 is important for *gcg* and GLP-1 production, we tested whether the defect in PTT in aged *Pak1*$^{-/-}$ mice is due to impairment in GLP-1. Aged *Pak1*$^{-/-}$ receiving forskolin injections, known to stimulate *gcg*, had elevated *gcg* expression associated with improved PTT. Furthermore, aged *Pak1*$^{-/-}$ mice receiving the DPP-IV inhibitor sitagliptin showed increased circulating GLP-1 levels, associated with complete reversal of the PTT defect and improved oral glucose tolerance. These results suggest that Pak1 stimulates glucose production *in vitro*, whereas the defect in whole-body pyruvate tolerance of is due to impairment in GLP-1. This study demonstrates the important role of Pak1 in liver glucose metabolism and homeostasis, and also advances our knowledge regarding the hepatic effects of the current incretin therapeutic sitagliptin.

E13. THE LOSS OF SirT1 IN PANCREATIC BETA CELLS IMPAIRS INSULIN SECRETION BY DISRUPTING GLUCOSE SENSING

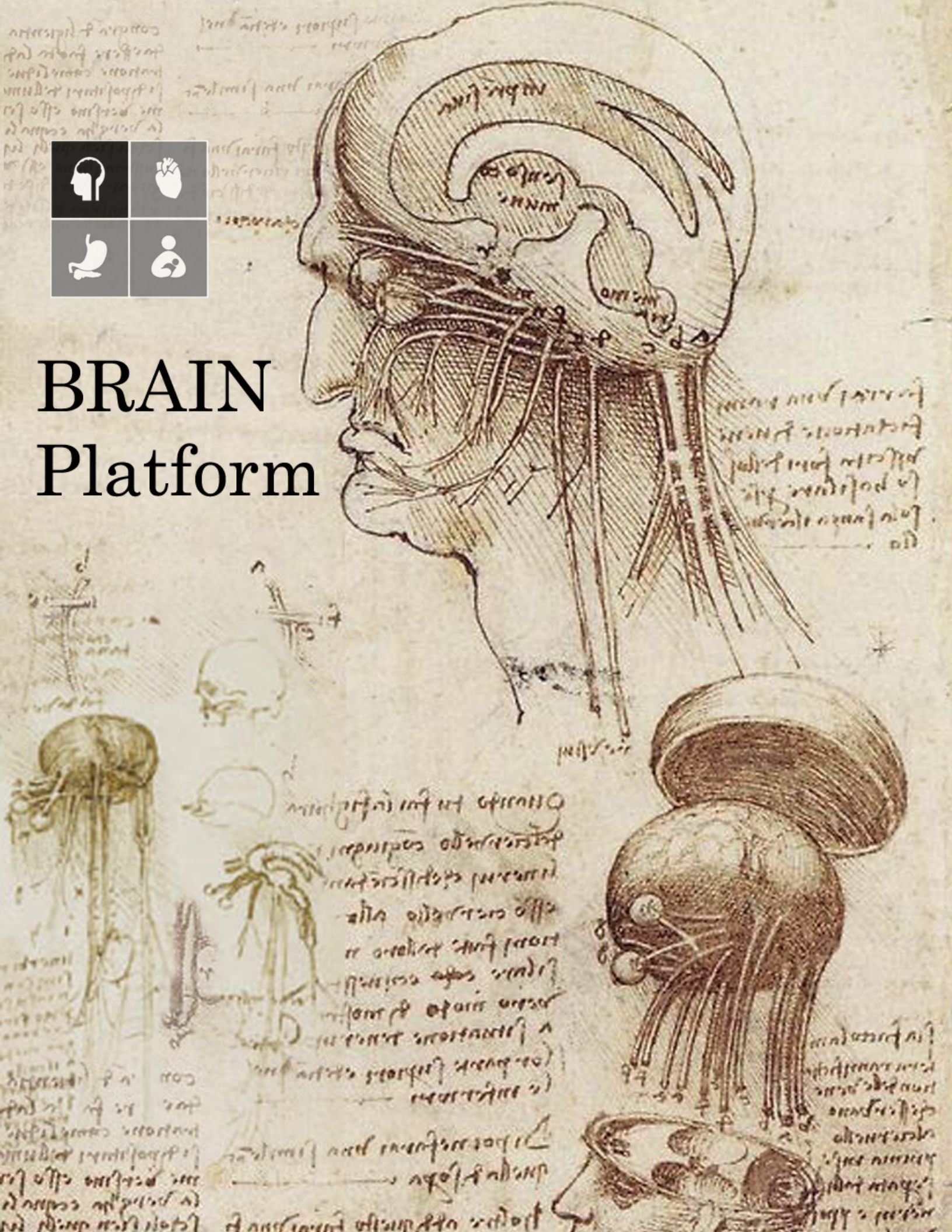
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SirT1 functions as an (NAD)-dependent deacetylase and is involved in the regulation of cell metabolism. Previous studies have shown that SirT1 is a positive regulator of insulin secretion and conferred protection against diabetes in mice. These findings suggest that SirT1dysfunction is involved in the etiology of diabetes although the precise mechanism is largely unknown. Using the inducible CreLox system, we aim to metabolically characterize a novel, inducible pancreatic beta cell-specific SirT1 knockout mouse (SirT1BKO) and determine the effect of SirT1 deletion on beta cell function. When orally challenged with glucose, SirT1BKO mice displayed glucose intolerance which correlated with decreased insulin secretion. Isolated SirT1BKO islets secreted less insulin (6.2±0.6 ng per 10 islets vs. 10.0±0.9 control) at 20mM glucose. Islets were assessed for beta cell mass and insulin content however no significant difference was observed suggesting reduced insulin secretion is due to an intracellular defect. When islets were stimulated with KIC (a substrate for the TCA cycle) SirT1BKO cells still secreted less insulin indicating the defects are downstream of glycolysis. Distally, SirT1BKO cells exhibited normal insulin granule exocytosis revealed by capacitance measurements. Interestingly, preceding this step, SirT1BKO cells displayed decreased influx of calcium at 20mM glucose. This may reflect defects upstream, namely mitochondrial metabolism. SirT1BKO cells displayed a 38±2.1% decrease in glucose-induced hyperpolarization compared to controls by measuring mitochondrial membrane potential, indicating the proton motive force is reduced. Furthermore, SirT1 knockdown MIN6 cells (SirT1KD) display a marked reduction in mitochondrial oxygen consumption rate. To gain mechanistic insight, SirT1KD cells resulted in a panel of 37 mitochondrial-related genes whose expression was significantly altered thereby disrupting mitochondrial dynamics ultimately affecting insulin secretion. These data highlight SirT1's role in insulin secretion and its potential for therapeutic use in diabetic patients.

E14. ACTIVATION OF AMP-ACTIVATED PROTEIN KINASE (AMPK) WITH 5-AMINOIMIDAZOLE-4-CARBOXAMIDE RIBONUCLEOTIDE (AICAR) ALLEVIATES CELLULAR INSULIN RESISTANCE IN NOVEL IMMORTALIZED HYPOTHALAMIC NEURONS

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Obesity is a major global health concern and is a major risk factor for other disorders, such as type 2 diabetes (T2D). Dysregulation of insulin signaling, both in peripheral tissues and at the cellular level in the hypothalamus, can lead to insulin resistance. In an obese state, insulin levels remain elevated, which can further reduce insulin sensitivity. Activation of AMP-activated protein kinase (AMPK), a key indicator of energy status, has been shown to restore insulin signaling in the periphery. However, its actions on insulin resistance in the hypothalamus are unclear. In order to further understand the role of AMPK at the level of the hypothalamus, we generated novel immortalized, hypothalamic cell lines, rHypoE-19 and mHypoA-NPY/GFP. Using semi-quantitative RT-PCR, we found that these neuronal cells express the endogenous insulin receptor (IR), as well as the proper insulin signaling molecules. We then created a model of cellular insulin resistance through chronic exposure of insulin, which was then followed by an insulin re-challenge to assess insulin signaling. After insulin pre-exposure, insulin signaling was attenuated upon 10 nM re-challenge, as assessed by attenuated Akt and hyperactivated S6K1 phosphorylation. We found that 1 mM pre-treatment with the AMPK activator, AICAR, was able to ameliorate chronic insulin-induced insulin resistance in both the rHypoE-19 and mHypoA-NPY/GFP cell lines. Here we show, for the first time, that activation of the AMPK pathway can reverse the effects of cellular insulin resistance at the cellular level in hypothalamic neurons. These studies provide an improved understanding of potential therapeutic targets to treat hypothalamic insulin resistance. (This research was supported by grants from BBDC, NSERC CIHR, CFI and CRC)



BRAIN Platform

THE DORSAL ROOT GANGLION SANDWICH SYNAPSE: NOVEL TRANSGLIAL SIGNALING BETWEEN NEURONAL SOMATA

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The dorsal root ganglion (DRG) contains a subset of closely-apposed neuronal somata (*NS*) that are separated solely by a thin satellite glial cell (*SGC*) membrane septum to form a *NS-glial cell-NS* (*NGlN*) cell trimer. We recently reported that stimulation of one *NS* evokes a delayed, noisy and long-duration inward current in both itself and its passive partner that was blocked by suramin, a general purinergic antagonist. Here we test the hypothesis that *NGlN* transmission involves purinergic activation of the *SGC* and its release of an excitatory transmitter. Stimulation of the NS triggered a sustained current noise in the *SGC*. Block of transmission through the *NGlN* by reactive blue 2 or thapsigargin, a Ca^{2+} store-depletion agent, implicated a Ca^{2+} store discharge-linked P2Y receptor. P2Y2 was identified by simulation of *NGlN*-like transmission by puff of UTP onto the *SGC*. Block of the UTP effect by BAPTA, an intracellular Ca^{2+} scavenger, supported the involvement of *SGC* Ca^{2+} stores in the signaling pathway. The response to UTP was also blocked by AP5, which, along with the NR2B subunit-specific antagonist ifenprodil, inhibited *NGlN* transmission, implicating a glutamatergic pathway via postsynaptic NMDA receptors. Puff of glutamate could evoke transmission-like current in the *NS*. Immunocytochemistry localized the NMDA receptor subunit NR2B to the *NS* membrane, abutting staining for P2Y2 on the *SGC* septum. We infer that *NGlN* transmission involves secretion of ATP from the *NS*, *SGC* Ca^{2+} store discharge via P2Y2 receptors and release of glutamate to activate NS postsynaptic NMDA receptors. Thus, the *NS* of the *NGlN* trimer communicate via a Sandwich Synapse transglial pathway, a novel signaling mechanism that may contribute to information transfer in other regions of the nervous system.

RECONCILIATION OF THE RECIPROCAL INTERACTION AND FLIP-FLOP MODELS OF RAPID EYE MOVEMENT SLEEP GENERATION

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Rapid eye movement (REM) sleep is one of the fundamental information processing states of the mammalian central nervous system. The seminal model of REM sleep generation, the reciprocal interaction hypothesis (RIH), postulates that the critical event in REM sleep induction is the cholinergic activation of the dorsal subcoeruleus region of the pons (SubCD). Alternatively, the flip-flop hypothesis (FFH), which maintains the SubCD as the putative site of REM sleep generation, posits that cycling into REM sleep critically involves the action of a mutually inhibitory GABAergic switch rather than a cholinergic mechanism. The necessity of cholinergic SubCD input for the generation of REM sleep is ultimately unknown as the critical test of this hypothesis has not been performed: local blockade of acetylcholine receptors in the SubCD. Using bilateral reverse microdialysis in freely behaving rats (n=18) chronically instrumented for electroencephalographic and electromyographic recording we locally manipulated neurotransmission at the SubCD. Microperfusion of an acetylcholine receptor antagonist (scopolamine, 1mM, n=14) into the SubCD - which completely prevents the effects of SubCD cholinergic agonism (carbachol, 0.5mM, n=4) - produced no significant effects on REM sleep quantity or frequency. This suggests that transmission of REM sleep drive to the SubCD is acetylcholine-independent contrary to the RIH. However, an analysis of moment-to-moment EEG spectral state changes showed that cholinergic SubCD inputs do: (i) positively reinforce transitioning into REM sleep once initiated and (ii) maintain stability of the REM sleep once transitions are complete. Antagonism of SubCD acetylcholine receptors increased: (i) non-REM to REM sleep transition duration and failure rate and (ii) spectral instability in REM sleep. Artificial circuit simulation showed that sharpening of state transitions observed *in vivo* can be modeled by connecting a positive feedback loop (i.e., cholinergic element of the RIH) to a mutually inhibitory bi-stable switch (i.e., switching element of the FFH).

GABAERGIC TROPHIC SIGNALING IN HIPPOCAMPAL NEUROGENESIS: EXAMINING THE IN-VITRO EFFECTS OF SELECTIVE $GABA_AR$ AGONISTS

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Adult neurogenesis (AN) in the mammalian hippocampus is a remarkable example of neural plasticity that occurs throughout life in distinct neurogenic microenvironments. One such region is the dentate gyrus (DG) in the hippocampus. Neural progenitor cells (NPCs) in the DG undergo a choreographed process while maturing into excitatory dentate granule cells (DGCs). GABA signaling through extrasynaptic $GABA_AR$ generates tonic depolarizing current in immature neurons, which is critical for the progression of NPCs into integrated, electrophysiologically viable, adult DGCs. Direct activation via $GABA_ARs$ exerts regulatory effects on proliferation, survival, and integration of DGCs. Hippocampal slice-cultures were used to characterize the action of THIP and investigate $\delta GABA_AR$ influence on hippocampal AN. The selective GABA agonist, THIP, acts with superagonist properties on δ-subunit-containing $GABA_AR$'s ($\delta GABA_AR$). Past experiments have shown THIP promotes increased neuronal survival and maturation in rat brain in vivo. The present study was initiated using hippocampal slice cultures from P7 rats. Hippocampal cultures were maintained for 2 weeks and upon analysis the characteristic morphological organization of the DG was shown to be intact. Application of the thymidine analog Chlorodeoxyuridine (CldU), and subsequent immunohistochemical staining for CldU and the immature neuronal marker Doublecortin (DCX) allows for quantification of new neuron production. Preliminary evidence suggests approximately 64% of $CldU^+$ cells are co-labeled with DCX, indicating they are newborn neurons. The mature neuronal marker, Calbindin, is also used to determine maturity of newborn DGCs in slice cultures. We hypothesize that THIP promotes neuronal maturation and survival by directly activating newborn DGCs in-vitro. Reduced neurogenesis impairs hippocampus-dependent learning and aberrant neurogenesis is involved in clinical conditions such as epilepsy, depression, and Alzheimer's. Understanding the influence of GABAergic signaling on the maturation and survival of adult-born DGCs in the hippocampus may lead to insights that guide novel therapies for neurological diseases. Supported by NSERC and CIHR.

B.R.A.I.N: Posters

B1. CHARACTERIZATION OF DENDRITIC SPINES IN RAT SPINAL CORD LAMINA I AND II NEURONS

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Dendritic spines serve not only as points of contact for presynaptic axons to communicate with the postsynaptic neuron, but also as computational units critical to synaptic plasticity. In the brain there are well-characterized differences in the presence and density of spines across different types of neurons. Here we studied dendritic spines in neurons in the superficial layers of the spinal dorsal horn. We used acute spinal cord slices from adult rats and filled neurons in lamina I and II with Lucifer yellow through a patch-clamp pipette. Slices were fixed with PFA, and imaged using 2-photon microscopy to build 3D reconstructions of filled neurons. Slices were then labelled for CGRP, IB4 and NeuN to confirm laminar localization. 21 of 23 neurons injected with Lucifer yellow were adequately filled and included for analysis. 6 of 11 lamina I neurons had spines, while 9 of 10 lamina II neurons had spines. On average, the density of spines in spiny lamina II neurons was greater than that of spiny lamina I neurons. Lamina I neurons were classified morphologically as fusiform (n=6), pyramidal (n=3) or multipolar (n=2). We found that one-third of fusiform, two-thirds of pyramidal and both multipolar neurons were spiny. Moreover, spine density of multipolar cells was greater than that of fusiform or pyramidal cells. These results suggest that in the superficial dorsal horn there are laminar and morphological cell-type differences in spine density. These differences may contribute to functional differences across populations of lamina I and II neurons.

B2. MECHANISTIC INSIGHTS INTO THE ROLE OF MUNC18 PROTEIN IN DEGRANULATION OF THE MAST CELLS

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Despite of its essential nature, the precise function of Munc18 protein in SNARE-mediated exocytosis in has been highly controversial. Recent analysis of patients with familial hemophagocytic lymphohistiocytosis type 5 has identified the E132A mutation in Munc18-2, prompting to examine the role of this protein in context of immune cell secretion. Lentivirus-mediated stable double knockdown of Munc18-1 and Munc18-2 in RBL-2H3 mast cell eliminated both IgE-dependent and ionomycin-induced granulation and caused significant reduction in syntaxin-11 level without altering expressions of other syntaxin isoforms. These phenotypes were effectively rescued upon reexpression of not only wild-type Munc18-2, but also Munc18-1, indicating there is no isoform specific functions among Munc18 proteins in mast cells. However, introduction of mutant forms (K46E, E59K, F115E or E132A) of Munc18 protein that have been implicated with deficits in syntaxin binding were unable to rescue the decreased degranulation and syntaxin-11 level. Indeed, these mutants show that they are unable to directly interact with syntaxin-11, tested through in vitro binding assay. Thus, this study demonstrates the crucial role of the Munc18 protein in mast cell degranulation. Mechanistically, Munc18 may participate in SNARE-mediated degranulation via its regulation of syntaxin-11.

B3. THE ROLE OF CAV2.2 C-TERMINUS IN CALCIUM CHANNEL ANCHORING AT THE PRESYNAPTIC TRANSMITTER RELEASE SITES

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Voltage gated neuronal calcium channels (Cav2.2) gate neurotransmitter release at specialized regions within the presynaptic nerve terminals, known as transmitter release sites (TRS). Studies have predicted that calcium channels are closely attached to the TRS in an organized structure, supporting a channel-channel anchoring mechanism. Although calcium channels have been linked to a broad range of presynaptic proteins, molecular components of the channel-channel anchor are poorly understood. Our objective is to identify the mechanism of Ca^{2+} channel anchoring at the TRS and will explore the role of channel cytoplasmic regions. GST or strep tagged Cav2.2 cytoplasmic region fusion proteins were used for pull-down assays from which distal C-terminus region ($C3_{strep}$) captured the channel from brain lysate. In order to determine the specific region responsible for Cav2.2 capture, GST fusion proteins comprising overlapping proximal ($C3_{proximal}$) and distal ($C3_{distal}$) regions of $C3_{strep}$ were generated. Our data show that $C3_{distal}$ region pulls down the channel from avian synaptosome membrane lysate. This finding was supported by a reduction of channel capture from brain lysate in the presence of a blocking peptide mimicking the last four amino acids of long splice variant Cav2.2 distal C-terminus which code a PDZ-ligand domain. $C3_{strep}$ protein was also able to pull-down transfected Cav2.2 from tsA201 cell lysate, narrowing down possibilities of a channel-channel bridging protein(s). Overall, these data suggest a key role for Cav2.2 distal C-terminus in channel anchoring at TRS.

B4. THE ROLE OF MITOCHONDRIAL DYSFUNCTION IN THE PATHOGENESIS OF RETT SYNDROME

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Rett syndrome (RTT) is a neurodevelopmental disorder affecting primarily females caused by mutations in the X-linked *MECP2* gene. Recent studies have identified impairments of oxidative phosphorylation and free radical metabolism, and also evidence of elevated neural oxidative stress indices in MeCP2 deficient tissues. These results lead to a mitochondrial hypothesis of RTT: mitochondrial impairment is a key factor in etiology of RTT. To date, however, this hypothesis remains largely correlative, as few studies have examined mitochondrial properties in MeCP2-deficient cells and no functional studies have been conducted on MeCP2-deficient neurons. We hypothesized *that mitochondrial function and morphology are altered in MeCP2 deficient cells, and that treatments to enhance mitochondrial function will ameliorate cellular deficits caused by lack of MeCP2.* We differentiated neurons from MeCP2-null mouse embryonic stem cells and controls from wild type stem cells. An array of fluorescent dyes coupled with confocal microscopy and/or flow cytometry was used to study mitochondrial function, morphology, and cell viability under basal condition. Western blot analysis was used to determine levels of mitochondrial proteins (e.g. mtHSP70, mtTFA, Sirt3, etc.) in whole cell lysates and mitochondria-enriched fractions. Under basal conditions 7 and 10 DIV MeCP2-null neurons displayed increased ROS levels and increased mitochondrial membrane potential when compared to wild type (WT). No differences were observed in cell death and mitochondrial mass between MeCP2-null and WT. Western blot analysis revealed that levels of Sirt3 and mtHSP70 were decreased in mitochondrial enriched fraction in MeCP2 null cells compared to WT. Trafficking of mitochondria along the neurites was also slowed down in MeCP2-null cells. Taken together these data indicate functional changes in neuronal mitochondria in the model of RTT. Since 99% of mitochondrial proteins are nuclear encoded and imported in mitochondria our future studies are focused on determining the import rates of mitochondrial proteins, as well, as on the developing rescue strategies.

B5. δGABA$_A$ RECEPTORS PROMOTE MEMORY AND NEUROGENESIS IN THE DENTATE GYRUS

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γ-Aminobutyric acid type A receptors that contain the delta subunit (δGABA$_A$Rs) are highly expressed in the dentate gyrus (DG), where they regulate neuronal activity. The DG is critically involved in memory and also exhibits significant neurogenesis, but the role of δGABA$_A$Rs in these processes is poorly understood. Accordingly, we sought to determine the role of δGABA$_A$Rs in learning and neurogenesis. Learning and neurogenesis were studied in wild-type (WT) mice and transgenic mice that lacked δGABA$_A$Rs (*Gabrd-/-*). To pharmacologically increase δGABA$_A$R activity, mice were treated with the δGABA$_A$R-preferring agonist 4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol (THIP). Behaviour was measured in recognition memory, contextual discrimination and fear extinction assays. Neurogenesis was studied by measuring the proliferation, survival, migration, maturation and dendritic complexity of adult-born neurons in the DG. *Gabrd-/-* mice exhibited impaired recognition memory, contextual discrimination and fear extinction relative to WT mice. Neurogenesis was disrupted in *Gabrd-/-* mice as the migration, maturation and dendritic development of adult-born neurons was impaired. Long-term treatment with THIP facilitated learning and neurogenesis in WT but not *Gabrd-/-* mice. δGABA$_A$Rs promote learning and neurogenesis, and can be pharmacologically targeted to enhance these processes.

B6. MECHANISMS OF SEPTIN 5-MEDIATED INHIBITION OF NEUROTRANSMITTER RELEASE

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Neurons communicate at chemical synapses via exocytosis of synaptic vesicles containing neurotransmitter. Exocytosis occurs when vesicle and plasma membranes fuse, a process mediated by the interaction of SNARE proteins. Protein interactions with SNARE proteins can therefore influence exocytosis. Septin 5, a filamentous cytoskeletal protein, binds the SNARE protein syntaxin 1A. Septin 5 is expressed predominantly in the brain where it associates with synaptic vesicles, prevents close docking of synaptic vesicles at the plasma membrane, and inhibits exocytosis. However, the specific mechanism underlying the inhibition of exocytosis by septin 5 is unknown. The current study aims to map the region(s) of septin 5 responsible for binding to syntaxin 1A. Intriguingly, two sequences found within septin 5 resemble sequences found in the SNARE-binding protein complexin. Once the binding regions have been characterized, mutant septin 5 lacking the binding region will be expressed in septin 5 -/- neurons to examine the role of this interaction in the regulation of SNARE mediated neurotransmission. This study will provide important advances in our understanding of the mechanisms regulating exocytosis and neurotransmitter release.

B7. SYNAPTIC VESICLE CAPTURE BY INTACT CAV2.2 CHANNELS

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The fusion of synaptic vesicles (SVs) at the presynaptic transmitter release face is gated by Ca^{2+} influx from nearby voltage gated calcium channels (CaV). Our early functional studies argued that the CaV and SV are linked by a molecular anchor or 'tether' and recent studies have proposed a direct cytoplasmic link to the channel distal C terminal. In order to explore CaV-SV binding we developed an *in vitro* assay, termed SV-PD, to test for capture of purified, intact SVs. Antibody-immobilized presynaptic or expressed CaV2.2 channels but not plain beads, IgG or pre-blocked antibody successfully captured SVs, as assessed by Western blot for a variety of protein markers. SV-PD was also observed with a distal C terminal fusion protein, $C3_{strep}$, supporting involvement of this CaV region. Our results favor the model where presynaptic CaV can tether SVs directly, independently of the surface membrane.

B8. THE CONTINUOUS UPREGULATION OF GABA$_A$ RECEPTORS AND TONIC INHIBITION BY ISOFLURANE AND ETOMIDATE

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Many patients assume that cognitive function rapidly returns to baseline after general anesthesia, but we showed that commonly used anesthetics, isoflurane (ISO) and etomidate (ETM) causes memory deficits for at least 24 hours in wild-type (WT) mice post-anesthesia. g-aminobutyric acid type A receptors (GABA$_A$Rs) that contain a5 subunit (a5GABA$_A$Rs) might contribute to such memory deficits, since pharmacological inhibition of a5GABA$_A$Rs prevents these deficits. Hence, we postulate that a5GABA$_A$Rs might be persistently up-regulated long after general anesthesia. To test this, mice were treated with either an inhaled anesthetic ISO or an intravenous anesthetic ETM, and 24 hours later, hippocampal tissue was obtained. Surface protein expression and mRNA levels were probed for GABA$_A$R subunits with biotinylation followed by western blot and quantitative RT-PCR, respectively. The surface expression of b2/3 subunits, which partner with a5 subunit, increased 24 hours after ISO and ETM treatment. The surface expression of a5 subunit also increased 24 hours after ISO treatment. However, mRNA levels of the a5 subunit remained unchanged after ISO and ETM. Thus, ISO and ETM cause an up-regulation in the expression of GABA$_A$Rs, that persists long after the anesthetics have been eliminated, which might not result from increases in translation. We are currently examining whether: (1) surface expression of a5 and other GABA$_A$Rs subunits changes 24 hours after ETM treatment, and (2) the persistent increase in a5GABA$_A$Rs results from increase in exocytosis or decrease in endocytosis.

B9. THE ROLE OF RGMA AND NEOGENIN IN EMBRYONIC CHICK SPINAL CORD DEVELOPMENT

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In the developing spinal cord, growing commissural axons travel along a complex path in which they are guided towards the ventral midline, cross towards the contralateral side, turn rostrally and are guided along the longitudinal axis - a process which requires the coordination of multiple guidance cues. The Repulsive Guidance Molecule a (RGMa) contributes to retino-tectal axon guidance in the developing chick brain and this effect is mediated by its transmembrane receptor Neogenin. It is however largely unknown whether these proteins play a role in axon guidance in the developing spinal cord. Studies were initiated by performing *In Situ hybridization* experiments on cyrosections of the chick spinal cord using digoxygenin labeled riboprobes against RGMa and Neogenin. These experiments revealed that the mRNA of these proteins are expressed in the developing chick spinal cord in a spatial and temporal fashion suggestive of role(s) in commissural neuron axon development. Synthetic miRNA constructs targeting chick RGMa and Neogenin were then generated and their effectiveness at knocking down these proteins were evaluated via western blotting. Spatially and temporally selective knockdown of these proteins via *in vivo* electroporation of miRNA constructs in the developing chick neural tube lead to abnormal axonal phenotypes of commissural neurons. Experiments are underway in which chick commissural neuron explants are grown on laminin alone or laminin plus N-RGMa or C-RGMa

and outgrowth is evaluated. Understanding of the functions of RGMa and Neogenin during spinal cord development can provide insight into how the developing CNS establishes correct neuronal wiring and can also provide clues on how to reestablish damaged connections in the injured adult CNS.

B10. δ-SUBUNIT CONTAINING $GABA_A$ RECEPTOR ACTIVATION IN THALAMOCORTICAL NEURONS SUPPORTS CORTICAL DEACTIVATION

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Gamma-amino butyric acid (GABA) is the main inhibitory neurotransmitter in the brain and augmentation of GABAergic neuronal activity promotes natural sleep. Many anesthetics enhance neural inhibition via interactions with binding sites on δ-subunit-containing $GABA_A$ receptors ($\delta GABA_AR$). The thalamus is a key structure controlling brain arousal and has a high density of $\delta GABA_AR$. Activation of $\delta GABA_AR$ in the ventral basal complex (VBc) of the thalamus elicits a tonic hyperpolarization in vitro which promotes a change in cell population firing patterns that is consistent with brain deactivation. . We examined the influence of $\delta GABA_AR$ activity in the VBc on cortical activity. Experiments were conducted in 25 freely behaving $\delta GABA_AR$ knockout ($Gabrd^{-/-}$) and wild type (WT) mice. Electrocortical and postural muscle activities were recorded during bilateral microperfusion of artificial cerebrospinal fluid (aCSF), 10μM, and 50μM THIP (a $\delta GABA_AR$-preferring agonist) into the VBc. Mice of each genotype also served as time controls, receiving only aCSF. In WT mice, 50μM THIP at the VBc increased 1-4Hz electrocortical activity in non-rapid eye movement (NREM) sleep and waking ($p<0.05$). Transitions into NREM and REM sleep occurred more rapidly with 50μM THIP treatment ($p<0.05$). Sigma power (10-15Hz) and spindle density (7-14Hz) during NREM sleep were also reduced ($p<0.05$). Importantly, no such changes occurred with THIP in $Gabrd^{-/-}$ and WT time-control mice. The presence of prominent delta oscillations (1-4Hz) in electrocortical activity is considered a hallmark of both sleep and anesthesia. Enhancing tonic inhibition through $\delta GABA_AR$ in the VBc would promote hyperpolarization of thalamocortical neurons and changes in neural activity that are consistent with brain deactivation. This prediction is supported through our data. We also identified an increase in the speed of transitions into NREM and REM sleep, indicating that delivery of THIP into the VBc promotes sleep induction.

B11. POTENTIATION OF GABAA RECEPTOR ACTIVITY BY VOLATILE ANESTHETICS IS REDUCED BY α5GABAA RECEPTOR-PREFERRING INVERSE AGONISTS

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Background: General anaesthetics trigger memory deficits that persist long after the drugs have been eliminated. Animal studies have shown that memory deficits in the early post-anesthetic period can be prevented by pre-treatment with an inverse agonist that preferentially inhibits α5 subunit–containing γ-aminobutyric acid type A (α5GABAA) receptors. The goal of this in vitro study was to determine whether inverse agonists that inhibit α5GABAA receptors reduce anesthetic potentiation of GABAA receptor activity. Methods. All experiments were approved by the local ethics review committee. Cultures of hippocampal neurons were prepared from Swiss white mice, wild-type mice and α5GABAA receptor null mutant (Gabra5–/–) mice. Whole-cell voltage clamp techniques were used to study the effects of the α5GABAA receptor–preferring inverse agonists, L-655,708 and MRK-016, on anaesthetic potentiation of GABA-evoked currents. Results. L-655,708 reduced sevoflurane potentiation of GABA-evoked current in wild-type neurons but not Gabra5–/– neurons, and produced a rightward shift in the sevoflurane concentration–response plot (sevoflurane EC50: 1.9±0.1 mM; sevoflurane + L-655,70850 nM EC50: 2.4±0.2 mM, $P<0.05$). Similarly, L-655,078 reduced isoflurane potentiation of GABA-evoked current (isoflurane: 4.0±0.6 pA/pF; isoflurane + L-655,70850 nM: 3.1±0.5 pA/pF, $P<0.01$). MRK-016 also reduced sevoflurane and isoflurane enhancement of GABA-evoked current (sevoflurane: 1.5±0.1 pA/pF; sevoflurane + MRK-01610 nM: 1.2±0.1 pA/pF, $P<0.05$; isoflurane: 3.5±0.3 pA/pF; isoflurane + MRK-0161 nM: 2.9±0.2 pA/pF, $P<0.05$). Conclusions. L-655,708 and MRK-016 reduced the potentiation by inhaled anesthetics of GABAA receptor activated by low concentration of GABA. Future studies are required to determine whether this effect contributes to the memory preserving properties of inverse agonists after anesthesia.

B12. MATHEMATICAL MODELS OF INHIBITORY NETWORKS PREDICT POSSIBLE MECHANISMS UNDERLYING HIGH FREQUNCY RHYTHMS IN THE HIPPOCAMPUS

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Hippocampal high frequency oscillations (HFOs: >100 Hz) have been proposed to play an important role in decision making and memory processing. These rhythms are found nested in slower theta rhythms (4-12 Hz) during REM sleep and spatial navigation of rats, but the mechanism(s) by which they are generated remain unclear. We use mathematical models to investigate the conditions under which parvalbumin-positive (PV+) fast-firing inhibitory cell networks can produce these high frequency oscillations. Experimental data from an intact hippocampus *in vitro* was used to obtain a clear biological context. Importantly, cellular characteristics and the amount of input that these cells receive during an ongoing theta rhythm was estimated and used to constrain our models. Each cell in the network received excitatory input and synaptic inhibition from presynaptic interneurons, and were systematically varied within experimentally determined ranges. For each combination of these values, the coherence of the network population firing and the network frequency was determined. For each of these network simulation sets, we also varied the connectivity probability and network size to explore how they affect the network's ability to produce coherent firing. We find that when our connection probability is experimentally constrained, our networks exhibit a sharp transition from random firing to network coherence with only a small change in synaptic input. However, as connectivity in the network is increased (or as network size is decreased) beyond experimentally estimated values, a larger window of coherence is achieved with a smooth transition from random to coherent firing. Our work indicates that fast-spiking PV+ networks can produce high frequency population rhythms, and that perturbation in and out of coherent states can occur abruptly. We propose that gating in and out of coherence could underlie mechanisms controlling the generation of HFOs in hippocampal circuits.

B13. THE ROLE OF PAK SIGNALING IN SYNAPTIC TRANSMISSION AND PLASTICITY USING A TETRACYCLINE INDUCIBLE SYSTEM IN MICE

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Neurodevelopmental disorders including autism, Alzheimer's disease and intellectual disability are among the most devastating deficits of mental and neurological diseases and are currently the leading socioeconomic health-care problems. These brain diseases are associated with a diversity of potential causes, including abnormalities in single gene mutations. PAKs (p21-activated kinases) 1-3 are a family of serine/ threonine protein kinases that are target enzymes of Rho small family GTPases and central regulators of actin cytoskeleton and neuronal morphology. *In vivo* studies reveal that PAKs are involved in synaptic and behavioural plasticity, including dendritic

spine morphology and learning and memory. Mutations in the PAK gene are implicated in various brain diseases however we do not understand how these mutations cause synaptic and behavioural deficit. We employ a tetracycline inducible system where the dominant negative PAK3 mutation can be spatiotemporally modulated. To evaluate cognitive performance, we conducted Morris water maze and fear conditioning tests and found that mutant PAK3 mice had profound impairments in both short and long term spatial and associative memory. Furthermore, the learning deficit in the mutant mice can be rescued with the administration of a tetracycline analog that blocks the expression of the mutant PAK3 transgene, which suggests that the memory impairments are not perturbed at development and are caused by deficits in mature synapses. Accordingly, we showed that mutant mice had reduced basal synaptic strength and plasticity that were not due to alterations in presynaptic function. Our data indicate that the molecular pathways through which PAK3 may mediate the Rho signaling process through cofilin dependent actin regulation in the cortex and hippocampus has a central role in the regulation of cognitive and synaptic function.

B14. THE DOMAIN-3A OF Munc18-1 PLAYS A CRUCIAL ROLE AT THE PRIMING STAGE OF EXOCYTOSIS

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Brain function depends critically on synaptic communication which is mediated by the release of neurotransmitters (exocytosis). Munc18-1 is an indispensible protein that highly regulates exocytosis by specifically interacting with one or more SNARE proteins (syntaxin-1, SNAP-25, and VAMP-2). Multiple lines of evidence have suggested the pleiotropic functions of Munc18-1: as a molecular chaperone of syntaxin-1, in priming of dense core vesicles (DCVs) to fusion competent state, and in docking of DCVs to the plasma membrane. Munc18-1 consists of multiple domains: domain-1, -2, -3a, and -3b. However, it is unclear how its structure and interactive properties contribute to its essential roles in exocytosis. We hypothesize that different domains of Munc18-1 selectively contributes to the specific functions through distinctive binding modes with SNARE proteins. To investigate this, we have stably re-expressed a series of domain-specific mutants in Munc18-1/-2 double knockdown PC12 cells through lentivirus mediated infection followed by detailed phenotypic analyses. In the course of analyzing the phenotypes of a variety of point mutations in domain-3a of Munc18-1, we discovered insertion mutants (K332E/K333E with insertions of 5 or 39 residues) that support the priming function of Munc18-1. These mutants completely lose their ability to rescue secretion while effectively restoring syntaxin-1 expression at the plasma membrane as well as DCV docking in Munc18-1/2 double knockdown PC12 cells. The mutants can bind syntaxin-1A but impairs binding to the SNARE complex compared to the wild-type. Our results advocate that the domain-3a of Munc18-1 plays a crucial role in priming of exocytosis by regulating SNARE complex interaction which is independent of its syntaxin-1 chaperoning and is downstream of dense-core vesicle docking. Our findings will provide novel insight into the obscure mechanisms underlying Munc18-1-regulated exocytosis.

B15. CONTRASTING DIFFERENCES IN NEURONAL MATURATION BETWEEN WILLIAMS-BEUREN SYNDROME AND 7q11.23 DUPLICATION SYNDROME MODELS

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Williams-Beuren syndrome (WBS) and 7q11.23 Duplication syndrome are neuro-developmental disorders caused by the deletion and duplication, respectively, of 26 genes on chromosome 7 including the general transcription factor 2I, GTF2I. The syndromes are both associated with neurocognitive and behavioral features with contrasting phenotypes. To test the role of duplication or deletion of this gene, Osborne's group previously generated mice with decreased (*Gtf2i*^{Del}) or increased (*Gtf2i*^{Dup}) genomic copy number of *Gtf2i*, a strong candidate for the neurobehavioral features of these disorders. The copy number of this gene has been linked to separation anxiety in both mice and humans. Thus, *Gtf2i*^{Del} and *Gtf2i*^{Dup} provide a useful model for understanding the molecular basis of both the 7q11.23 disorders and anxiety. TFII-I negatively regulates membrane targeting of TRPC3 by competing with TRPC3 for binding to phospholipase C-γ. These findings suggest a role for TFII-I as a negative regulator of agonist-induced calcium entry (ACE), which may be associated with the cognitive defects of WBS. However, the cellular effects of TFII-I deletion and duplication have not been tested. In this study we investigated the regulatory function of TFII-I on TRPC3 channels and neuronal morphology in vitro in our *Gtf2i*^{Dup} and *Gtf2i*^{Del} mouse models. Using primary cell culture we found significant differences in total neurite length and axonal branching between and *Gtf2i*^{Dup}, *Gtf2i*^{Del} and their wild type (WT) siblings. Furthermore we found differential distribution of TRPC3 channels in cell bodies, neurites and growth cones in the three groups supporting the notion that TFII-I regulates the cellular localization of the TRPC3 channel. Interestingly, we show differences in ACE. Thus, TFII-I regulated ACE may play a critical role in neuronal maturation in the cortical region. Together our results using the genetic models provide functional insight into the cellular mechanisms of the 7q11.23 syndromic disorders and perhaps anxiety disorders.

B16. CRYOLOADING: A NOVEL METHOD TO INTRODUCE ALIEN COMPOUNDS INTO ISOLATED FUNCTIONAL SYNAPTIC NERVE TERMINALS.

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The release of neurotransmitter from the presynaptic nerve terminals is achieved by the fusion of neurotransmitter-loaded synaptic vesicles (SVs) with the surface membrane in response to depolarization-induced Ca^{2+} influx (Katz and Miledi, 1967). Continued rounds of neurotransmitter release require the subsequent recovery of the fused SVs via endocytosis (Holtzman *et al.,* 1971). These events, termed the SV cycle, are mediated by a complicated network of protein-protein interactions, many of which are yet to be characterized. Specific disruption of these interactions and analysis of their effect on fusion or endocytosis in synaptosomes (SSMs) has been the primary functional method by which to identify and assess the roles of proteins. To this end, genetic manipulation, siRNAs, and membrane-permeant compounds have been used. These methods, however, are resource and time intensive and not widely applicable. Here, we present a novel, efficient and repeatable method by which to introduce virtually any alien compounds into isolated synaptic terminals—which, crucially, remain viable. The method relies on the phenomenon of freeze-thaw permeabilization, and as such we call it "cryoloading". Frozen cryoloaded SSMs can be stored indefinitely, allowing for the generation of libraries of variably-loaded SSMs. Using chick SSMs, we show that SSMs remain functionally competent following cryoloading (of a marker) and long-term storage—as evidenced by their ability to endocytose styryl dyes. Cadmium or ω-conotoxin addition to the extracellular solution indicates that cryoloaded SSMs require Ca^{2+} influx to proceed through the SV cycle, as expected. Cryoloading of a membrane-impermeant variant of the fast Ca^{2+} scavenger BAPTA prevents dye uptake, indicating successful cryoloading. Thus, the cryoloading method represents a simple, reliable and effective method to test effects of large test molecules on the function of normal brain presynaptic terminals. (This work was supported by a CIHR grant to EFS and an OGS grant for ARN.)

B17. NOVEL MOLLUSCAN PROTEIN SHOWS NEUROPROTECTIVE EFFECTS IN MPP$^+$-INDUCED PARKINSON'S DISEASE MODEL OF PC12 CELLS

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Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting approximately 1% of the population over the age of 60 and 4% of the population over 80. The principal basis of PD is the degeneration of dopaminergic cells in the substantia nigra pars compacta (SNpc). Currently, there is neither a definitive cure for PD nor a treatment available to slow down the degeneration of SNpc dopamine-containing neurons. Existing management strategies, such as monoamine oxidase-B inhibitors (MAOBIs), Levodopa, or dopamine agonists decline in effectiveness as PD progresses and may cause a range of side effects. This pathology can be modeled experimentally by *in vitro* administration of 1-methyl-4-phenylpyridinium (MPP^+), a highly toxic heroin analogue that induces cell death through inhibition of complex I of the mitochondrial electron transport chain and subsequent obstruction of ATP production. Some evidence also implicates MPP^+ in microtubule dysfunction, production of Lewy bodies and free radicals. This study demonstrates that a novel molluscan calcium-binding protein, ubiquitously expressed in central neurons of *Lymnaea stagnalis*, but with no mammalian homologues or orthologues provides neuroprotection in MPP^+-induced PD model in undifferentiated PC12 cells. Cultured PC12 cells were treated with only MPP^+ or MPP^+ together with novel protein, positive or negative controls. Both quantitative and qualitative cell viability assays revealed a significant decrease of cell death in cells treated with MPP^+ together with the novel protein compared to cells treated with only MPP^+ or MPP^+ together with positive or negative controls. This study may implicate this novel molluscan peptide as a potential strategy for one of the world's most widespread neurodegenerative diseases.

B18. ROLE OF VOLUME-REGULATED ANION CHANNEL (VRAC) IN NEONATAL HYPOXIC-ISCHEMIC INJURY

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Swelling-induced activation of Volume Regulated Anion Channels (VRACs) during ischemia mediates excitatory amino-acid (EAA) release and takes part in ischemic-induced damage. In this project, we evaluate the role of these channels in neonatal hypoxic-ischemic injury model using a selective VRAC blocker which is an ethacrynic acid derivative, 4-(2-butyl-6,7-dichloro-2-cyclopentyl-indan-1-on5-y1) oxobutyric acid (DCPIB). Previously, DCPIB demonstrated a promising neuroprotective effect in adult brain ischemia only when given intracisternally. Unlike adult animals with matured blood-brain-barrier (BBB) that prevents the blocker from entering the brain when given intravenously, perinatal and neonatal animals have immature formations of BBB. Hence, the intravenous or intraperitoneal application of DCPIB in these young animals may even be sufficient to provide neuroprotection during hypoxic-ischemic conditions. In this study, cerebral hypoxic-ischemic injury was induced in seven-day-old mouse pups, and DCPIB-treated mice showed a significant reduction in hemispheric corrected infarct volume (26.65 ± 2.23%) compared to that of the vehicle-treated mice (45.52 ± 1.45%) ($P<0.001$, n=5/group). DCPIB-treated mice also showed better functional recovery as they were more active than vehicle-treated mice at 4 and 24-hour post injury. In addition, DCPIB also reduced the OGD-induced cell death in vitro. These experiments further support the pathophysiological role of VRACs in ischemic brain injury, and suggest DCPIB as a potential, easily administrable therapeutic drug targeting VRACs in the context of perinatal and neonatal hypoxic-ischemic brain injury.

B19. POST-ANESTHETIC MEMORY DEFICITS ARE MEDIATED BY INCREASED ACTIVITY OF α5GABA$_A$RS

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General anesthetics cause memory deficits that persist long after the anesthetic has been eliminated. We previously showed that pharmacologically inhibiting γ-aminobutyric acid type A receptors that contain the α5 subunit (α5GABA$_A$R) both prevented and reversed memory deficits after anesthesia in mice (Anesthesiology 2010: 113 (5): 1061-71, Anes Analg 2012: 114 (4): 843-55). Here, we tested the hypothesis that α5GABA$_A$Rs are necessary for the development of anesthetic-induced memory deficits and that the activity of these receptors is enhanced after anesthesia. Wild-type (WT) and α5GABA$_A$R null-mutant (*Gabra5–/–)* mice were treated with the intravenous anesthetic etomidate (8 mg/kg i.p.). Memory was assessed 24 h, 72 h and 1 week later with the object recognition task. Also, mice were treated with etomidate and hippocampal slices were prepared 24 h later. Long-term plasticity was studied in the CA1 region after 20 Hz stimulation of Schaffer collaterals. To measure the activity of α5GABA$_A$Rs, the amplitude of a tonic inhibitory conductance generated by α5GABA$_A$Rs was measured in CA1 pyramidal neurons in brain slices using whole cell recording methods. The results showed that WT but not *Gabra5–/–* mice exhibited impaired recognition memory for at least 72 h after etomidate. In preliminary studies, long-term potentiation was reduced in brain slices from etomidate-treated WT mice and the amplitude of the tonic conductance was increased. Collectively these results suggest that α5GABA$_A$Rs are necessary for post-anesthetic memory deficits and these deficits may be caused by an increase in the activity of α5GABA$_A$Rs.

B20. EXAMINATION OF SYNAPTOSOMAL MEMBRANES THROUGH ELECTRON MICROSCOPY

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Isolated presynaptic terminals, known as synaptosomes (SSMs), have been used to investigate neurotransmitter release due to their retention of critical release machinery in a purified preparation. However, electron microscopy of SSMs for the purpose of investigating synaptic membrane and/or its associated structures is obstructed by the clutter of electron-dense components of the cytosol and the sheer concentration of organelles present. It is well established that synaptic vesicles can be expelled from the SSMs by osmotic shock/lysis, leaving an 'SSM ghost'. We are exploring the use of this preparation to examine presynaptic structures that remain associated with these ghosts and, hence, are likely attached to its surface membrane. As expected and shown previously, mitochondria and electron-dense cytosolic factors are lost while the postsynaptic apparatus remains attached to some of the SSM ghosts. While most of the synaptic vesicles are lost, a small fraction remains. We have also observed feathery structures protruding into the SSM ghost interior, which warrant further study. The specific method by which we generate these ghosts also allows us to immunogold label targets inside the ghosts without the use of permeabilizing agents such as saponin which visibly disrupts membranes. Thus, SSM ghosts provide an efficient model for investigating synaptic membrane-associated structures.

B21. *L*CABP, A NOVEL PUTATIVE CA2+-BINDING PROTEIN REQUIRED FOR LONG-TERM MEMORY FORMATION IN *LYMNAEA STAGNALIS*

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Neuronal calcium-binding proteins are critical to the activity-dependent modification of synaptic efficacy and neuronal excitability required for long-term memory (LTM) formation. Reflective of their importance, declines in calcium-binding protein expression has been shown to parallel aging and neurodegeneration-related memory deficits. Therefore, the identification and characterization of calcium-binding proteins involved in LTM formation is not only critical to furthering our understanding of the neural basis of memory formation, but also to the development of therapeutic tools. Recently, our lab has identified a novel EF-hand containing protein, *L*CaBP, that is required for LTM formation in an aversive operant conditioning paradigm of the freshwater pond snail *Lymnaea stagnalis.* Given previous

demonstration that *L*CaBP is a positive regulator of cAMP and CREB1 expression, we employed double-stranded siRNA knockdown and intracellular sharp electrode recording to examine the possibility that *L*CaBP may be a novel neuronal calcium sensor involved in the activity-dependent modification of neural activity required for LTM formation. We first confirm that *L*CaBP is indeed required for LTM formation after aversive operant conditioning. Subsequently, we demonstrate that LCaBP knockdown prevents the induction and/or expression of enhancement of both synaptic efficacy and neuronal excitability that underlie the LTM behavioural phenotype. Finally, we present evidence that *L*CaBP knockdown modifies RPeD1 action potential waveform in both naïve and conditioned animals. Together, the findings suggest *L*CaBP may indeed be a novel molecular player involved in the activity-dependent modification of neural activity during LTM formation in *L. stagnalis* that warrants further study.

B22. c-fos IMMUNOLABELING REVEALS CHANGES IN SOUND FREQUENCY REPRESENTATION IN AUDITORY MIDBRAIN FOLLOWING CHRONIC LOW-LEVEL NEONATAL SOUND EXPOSURE

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Background: Sensory areas of the brain have the remarkable ability to reorganize as a result of significant changes in peripheral sensory input, especially during early development. While cortex is often investigated as the primary site of developmental plasticity, our study provides evidence for midbrain plasticity. Our working hypothesis is that the development of auditory pathways is influenced in large part by patterns of sensory activation experienced during early development. Specifically, we hypothesize that post-natal exposure to an unusual sound environment modifies the neural representation of the exposure sound frequency in inferior colliculus (IC). Methods: Newborn chinchillas were reared in the presence of a chronic, moderately-intense (c. 70 dB SPL) narrowband sound signal (centered at 2 kHz) for 4 weeks. We estimated hearing thresholds using auditory brainstem responses (ABRs). We then observed neural activation patterns in IC using c-fos labeling (protocol optimization for chinchilla developed in-house, using commercially-available reagents). Results: Hearing thresholds were similar between control and sound-exposed subjects, suggesting that sound-exposure does not affect normal hearing. We observed a statistically-significant increase in the number of labeled cells both in the 2-kHz region (corresponding to the sound-exposure frequency) as well as throughout the IC of sound-exposed subjects, compared with controls. Conclusions: These results support the hypothesis that abnormal sound patterns at the periphery during a neonatal period can induce changes in neural activation patterns in sub-cortical auditory structures. We also discuss some plausible interpretations. Studying the effect of sound on the developmental plasticity of the auditory brain is important for our general understanding of how the auditory system develops. The work also has some relevance for the rehabilitation of the hearing impaired, for example with hearing aids and cochlear implants.

B23. CHOLINERGIC EXCITATION OF LAYER VI NEURONS IN CORTEX IS STRONGLY DEPENDENT ON CORTICAL REGION

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Cholinergic signalling in medial prefrontal cortex plays a key role in mediating normal attentional performance. Of particular interest, region specific expression of nicotinic α4β2* receptors and a variety of muscarinic receptors are found in layer VI pyramidal neurons across medial prefrontal cortex. However, neither the extent nor the nature of layer VI neuronal responses to acetylcholine have been contrasted across multiple cortical regions. Here, we investigated the responses to acetylcholine in layer VI neurons of three cortical regions (medial prefrontal cortex, mPFC; primary motor cortex, M1; and primary somatosensory cortex, SSC) using whole cell recording in acute brain slices. Acetylcholine elicited excitatory responses in layer VI neurons of all three cortical regions, with significantly greater effects in mPFC. Both nicotinic and muscarinic receptors contributed to the cortical cholinergic responses, with strongly region-dependent differences in the balance between these components. Pharmacological dissection of acetylcholine responses across regions showed a large and dominant α4β2* nicotinic receptor response in mPFC, but only a minor excitatory muscarinic component. In contrast, smaller acetylcholine responses in M1 and SSC were more balanced between their nicotinic and muscarinic components. Layer VI neurons in mice lacking the nicotinic receptor α5 accessory subunit do not exhibit these differences. Our results highlight the unique status of layer VI neurons of mPFC in terms of their unusually strong excitatory nicotinic response to acetylcholine that underlies their importance in attention.

B24. AN INVESTIGATION OF MOTOR CIRCUIT ACTIVITY PATTERNS UNDERLYING THE TRANSITIONS BETWEEN DIRECTIONAL MOVEMENTS IN *CAENORHABIDITIS ELEGANS*

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Locomotion is generated by neural networks that initiate rhythmic activity patterns entrained by sensory feedback. *Caenorhabditis elegans* serves an ideal model to dissect motor circuits with its simple nervous system yet complex behavioral patterns. The *C. elegans* locomotor circuit consists of two components: the forward- and backward-circuit dedicated to two directional movements. Receiving interneuron inputs, excitatory B- and A-motoneurons drive forward and backward movement, respectively. Inhibitory GABAergic D-motoneurons were proposed to relax the musculature opposing the contracted muscle segment, thus coordinating local body bending. Previously, our lab has shown that the activity difference between the forward- and backward-circuit outputs determines the directionality of locomotion. A key question remaining is how the nematodes execute directional movements and transitions through alternating the motoneuron activity patterns. Using GCaMP-based calcium-reporter lines and quantitative calcium-imaging, this study aims to elucidate how these motoneurons function together to generate coordinated locomotion. Previously, our lab found that during forward movements, a sequential activation of B motoneurons in an anterior-to-posterior direction drives body bend propagation. Using Ca^{2+} imaging, I observed that the activity pattern of A-motoneurons followed a posterior-to-anterior order during prolonged reversal. Surprisingly, during transitions from forward to backward movement, the initiation of A-motoneurons activity did not always start from the most posterior neuron as expected, but could start at any body segment. A putative proprioceptive property of the A-motoneurons is currently under investigation. In addition, I found the activity of D-motoneurons correlated with the local body bending and followed an anterior-to-posterior order during forward movement and a reversed order during backing, supporting its role in coordinating body bending in both directional movements and transitions. Therefore, we hypothesized that during transitions, the activity patterns effectively reset to shift the directionality of body bending propagation through the interaction between A- and D-motoneurons, which shall be tested in future experiments.

B25. THE ROLE OF DISRUPTED-IN-SCHIZOPHRENIA 1(DISC1), A KEY RISK FACTOR IN MAJOR PSYCHOLOGICAL DISORDERS, IN HIPPOCAMPAL SYNAPTIC PLASTICITY AND COGNITION

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Schizophrenia is a severe psychological disorder that affects about 1% of the population. One of the key characteristics of this disorder is a deficit in cognitive function such as learning and memory. Synaptic plasticity is a feature of the brain that is believed to be the basis for learning and memory and is represented by changes in synaptic strengths between presynaptic and postsynaptic neurons. Increases in postsynaptic response, termed 'potentiation', or decreases in this response, termed 'depression', underlie the mechanisms of synaptic plasticity and are often dependent on postsynaptic receptors such as N-methyl-D-aspartate receptors (NMDARs) and metabotropic glutamate receptors (mGluRs). Disrupted-in-Schizophrenia 1 (DISC1) has emerged as a strong genetic risk factor for psychological disorders such as schizophrenia, bipolar disorder, and major depression. The DISC1 protein consists of 854 amino acids and is known to interact with many proteins including Kalirin-7 (Kal-7), phosphodiesterase 4B (PDE4B), and glycogen synthase kinase-3b (GSK-3b). DISC1 has been found to be important in neurodevelopment through its involvement in neuronal proliferation and migration. This protein may also play a role in the regulation of synaptic plasticity as suggested by its interaction with proteins that are known to be involved in actin cytoskeleton regulation. For example, the guanine exchange factor, Kal-7, has been shown to play a role Rac1 signaling which is involved in actin cytoskeleton remodeling. Although DISC1 may potentially function in synaptic plasticity the exact mechanisms involved is still largely unexplored. In this project I will investigate, using DISC1 mutant mice (L100P and Q31L), whether DISC1 is important for the regulation of synaptic plasticity and to determine the molecular pathways involved in this regulation. I hypothesize that DISC1 is important for hippocampal synaptic plasticity acting through the Rac/Pak/Cofilin signaling pathway.

B26. CONFORMATIONAL SWITCH DETERMINES THE FUNCTION OF N-PEPTIDE OF SYNTAXIN-1

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The neuronal SNARE proteins VAMP2, SNAP-25, and syntaxin-1 form a complex that drives fusion of secretory vesicles with the plasma membrane in neurotransmitter release. Of the SNARE proteins, syntaxin-1 interacts with another essential synaptic protein Munc18-1 via two distinct modes: (i) "closed" conformation mode and (ii) N-peptide mode. These two modes have been thought to be mutually exclusive, but we investigate how these modes can complement each other to secure the binary interaction and contribute to exocytosis. Exocytosis of neurosecretory PC12 cells is markedly reduced through stable depletion of syntaxin-1A/1B but can be rescued upon re-expression of wild-type syntaxin-1A and more effectively by the "open" conformation mutant (L165A/E166A). Point mutations (D3R, L8A) or deletion (Δ20) of the N-peptide in wild-type ("closed") syntaxin-1 have little impact on its rescuing ability. However, these N-terminal mutations in syntaxin-1A adopting the "open" conformation profoundly inhibit its rescuing ability and aggravate its plasma membrane localization. Furthermore, biochemical experiments show that L8A or L165A/E166A mutation partially impairs the binding of syntaxin-1A to Munc18-1, whereas the combination of both mutations almost completely abolishes it. Our results uncover a striking interplay between the conformational state of syntaxin-1A and its N-peptide. We strongly argue that the two binding modes complement each other to ensure the proper trafficking and function of syntaxin-1.

B27. IDENTIFICATION OF KINASES INVOLVED IN THE PRO-APOPTOTIC STIMULUS OF NEOGENIN

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Axons of the adult central nervous system (CNS) show no functional recovery following injury. Several neurite outgrowth inhibitors have been associated with this phenomenon, such as myelin associated glycoproteins (MAG), oligodendrocytes-myelin glycoproteins (OMgp), and more recently Repulsive Guidance Molecule (RGMa). In addition to inhibiting axon outgrowth, RGMa acts to prevent cell death due to its interaction with a well-known dependence receptor, Neogenin. When unbound to their specific ligands, these receptors induce a state of apoptosis in the cells, which results in cell death. The pro-apoptotic activity of Neogenin is believed to be regulated by caspases. However, the kinases involved in this signaling pathway remain poorly understood. Here, it is hypothesized that, in its unbound state, Neogenin triggers a pro-apoptotic stimulus by activating specific intracellular kinases. Isolating these kinases will allow for a novel therapeutic target which will both rescue cell death and promote axonal regeneration following CNS injury. In order to isolate specific kinases involved in the downstream pro-apoptotic signaling cascade of Neogenin an inducible stable cell line expressing Neogenin will be generated. The expression of Neogenin will be monitored followed by silencing the expression of specific kinases in a kinome assay using an RNA-intereference technique. Cell viability will be assessed 48 hours post-transfection using cellTiter Blue. The elucidation of specific downstream Neogenin-induced pro-apoptotic kinases will provide a potent therapeutic target for drug development due to the availability of kinases inhibitors. Therapeutic agents targeting these specific kinases will potentially serve as a recovery treatment in CNS injury (i.e. stroke).

B28. DJ-1 DEFICIENCY INDUCES GENDER-SELECTIVE MODULATION OF BEHAVIOUR AND BIOGENIC AMINES IN MALE MICE

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Inactivating mutations in the DJ-1 gene cause a form of early-onset familial Parkinson's disease (PD). DJ-1 is also known to positively modulate androgen receptor activity through its interaction with PIASxα. It has long been appreciated that the incidence of PD in men is higher than in women. Factors that may contribute to this gender difference are of interest in establishing mechanisms of PD pathogenesis. We have now examined behavioural and neurochemical endpoints in male and female DJ-1-knockout (DJ-1-KO) mice. We found gender-selective increases in anxiety (zero maze), behavioural despair (tail suspension) and forelimb coordination (tape removal) in male DJ-1-KO mice. In contrast, object and spatial memory were modestly impaired in both male and female DJ1-KO mice. Tissue levels of dopamine, homovanillic acid, normetanephrine and 5-HIAA were selectively increased in discrete regions of the male DJ-1-KO brain. Male DJ-1-KO mice also exhibited levels of plasma testosterone that were elevated relative to wild type littermates. Our observations suggest that disrupted androgen signaling and high testosterone levels may contribute to gender-selective phenotypes of DJ-1-KO mice.

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