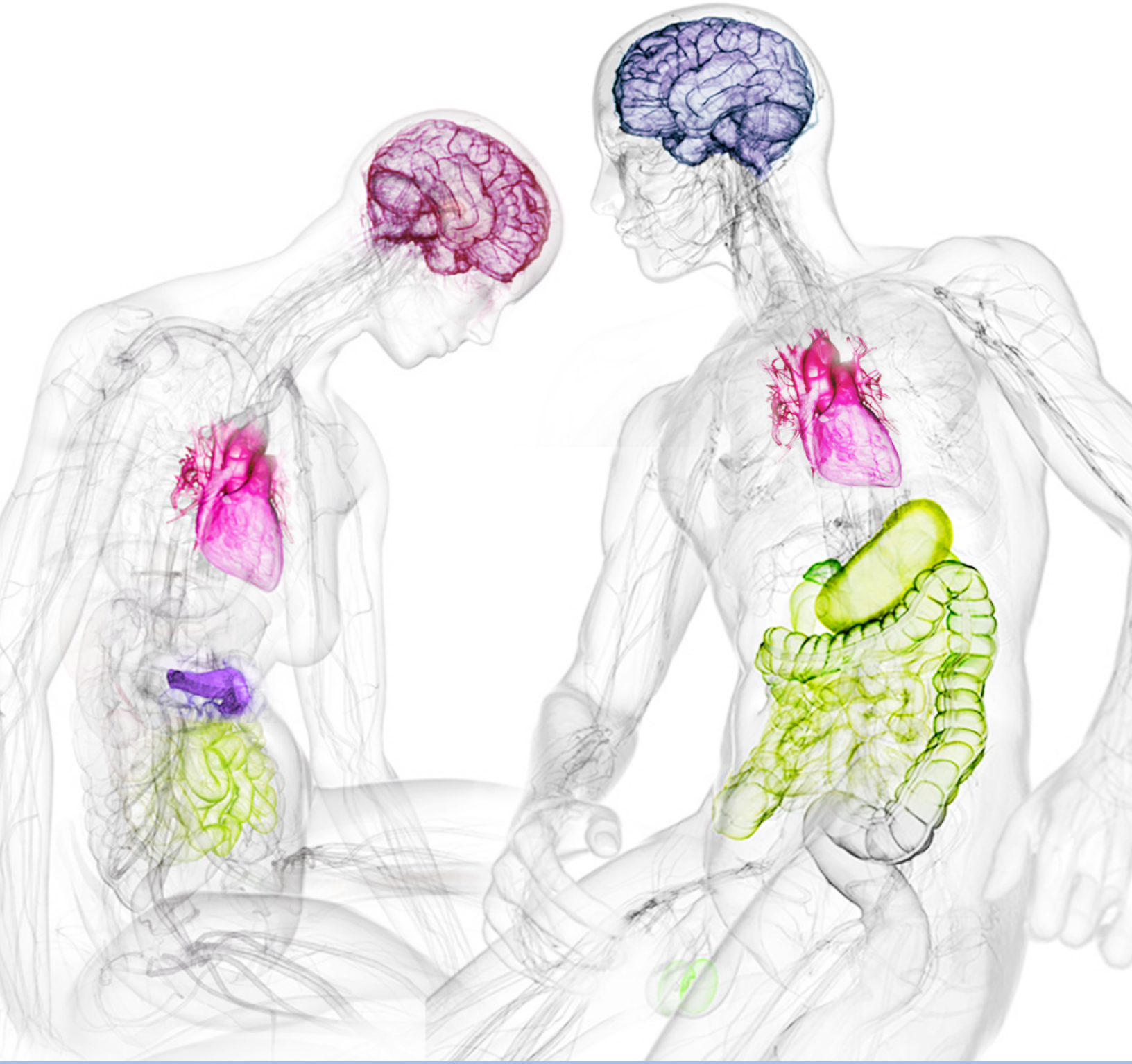
32nd Annual

FRONTIERS IN PHYSIOLOGY



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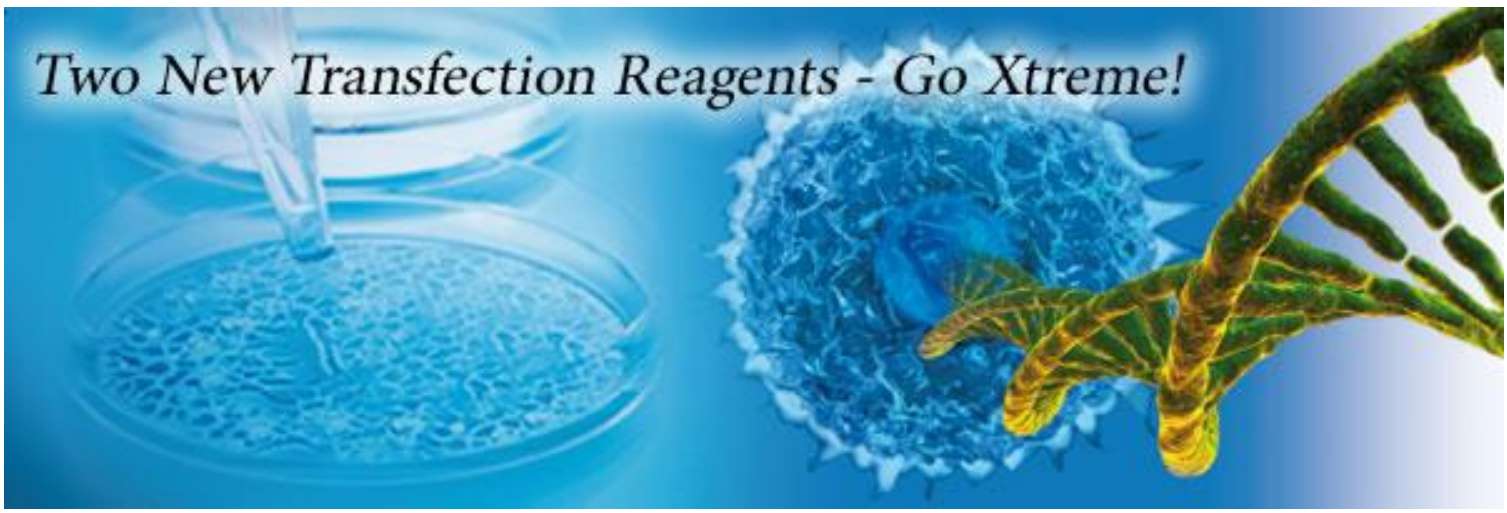
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Frontiers in Physiology Schedule

8:00 – 8:45 **Registration, Breakfast and Poster Setup** (Stone Lobby)

8:50 – 9:00 **Opening Remarks** – Majid Iqbal, Lemieux Luu and Dr. Stephen Matthews (MacLeod Auditorium)

9:00 – 9:45 **Cardiovascular Platform Oral Presentation** (MacLeod Auditorium)

M. Sauvé	The role of the tumor necrosis factor alpha/sphingosine-1-phosphate signalling axis in microvascular tone in a mouse model of type 2 diabetes mellitus.
R. Gao	Alpha 1-antitrypsin improves lung function in a rat lung transplantation model.
G. Bastin	Intracellular trafficking of regulator of g-protein signaling 4 (RGS4) requires palmitoylation and Rab family function.

9:45 – 10:00 **Break**

10:00 – 10:45 **B.R.A.I.N. Platform Oral Presentation** (MacLeod Auditorium)

R. Ferreira	Coupling of p2y-mediated store depletion & ca^{2+} entry (SOCE) to SK4 channels and microglia migration.
M. Lang	Preservation of MeCP2 in catecholaminergic cells is sufficient to improve life span and behavioural deficits in a mouse model of Rett Syndrome.
W. To	Systemic inflammation enhances general anesthetic endpoints.

10:45 – 11:00 **Break**

11:00 – 12:00 **KEYNOTE LECTURE** (MacLeod Auditorium)

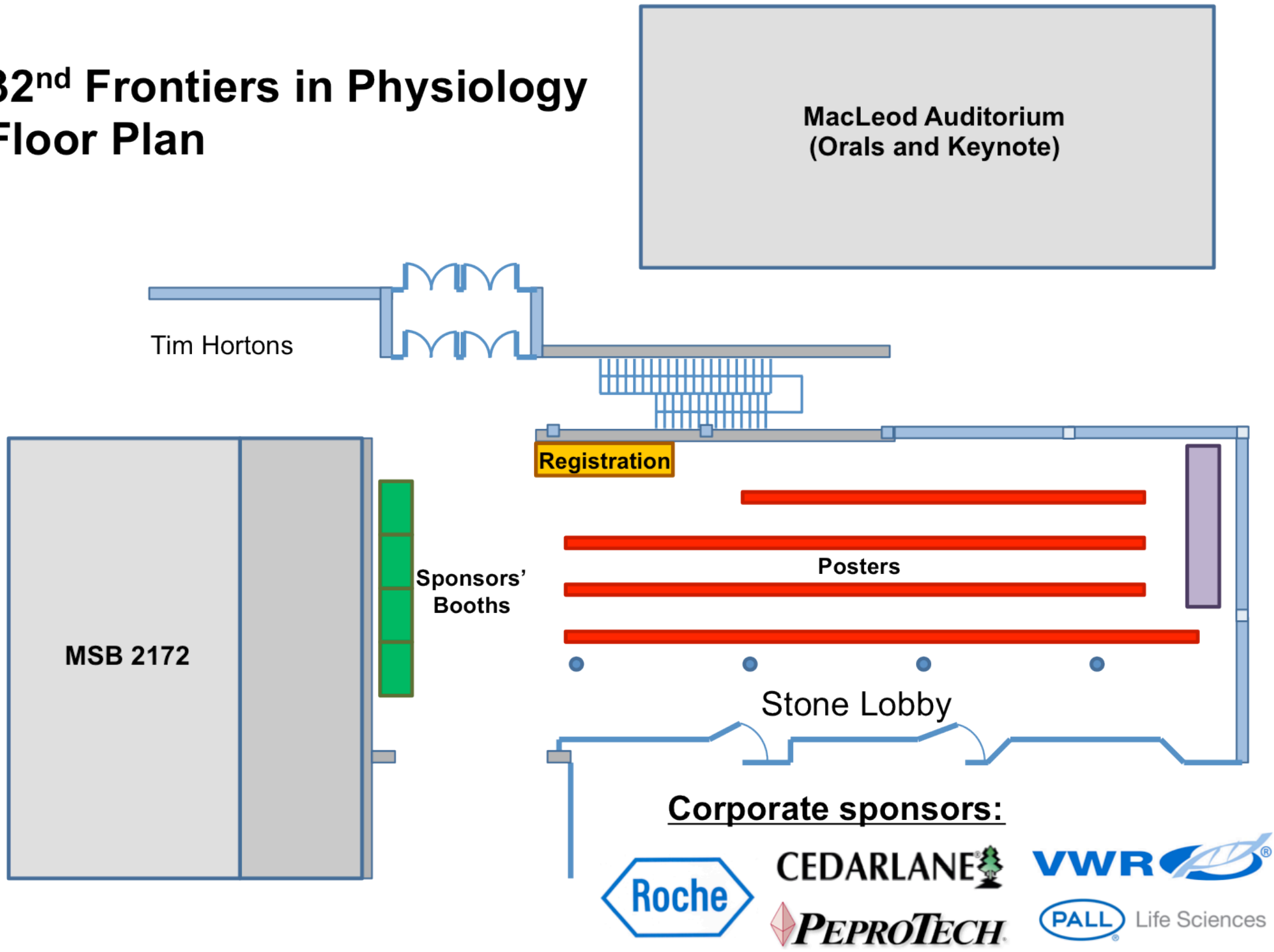
"Application of Optogenetics"

Karl Deisseroth, M.D., Ph.D.
Professor of Bioengineering and Psychiatry
Stanford University and Howard Hughes Medical Institute

Frontiers in Physiology Schedule (Cont'd)

12:00 – 12:45	**Poster Viewing and Lunch** (Stone Lobby)	
12:45 – 1:30	**Poster Judging** (Stone Lobby)	
1:30 – 2:00	**Departmental Photograph** (Outside Main Entrance)	
2:00 – 2:45	**Repro Platform Oral Presentation** (MacLeod Auditorium)	
	E. Fox	Signaling through FGFR2iiic is critical for murine embryonic stem cells and induced pluripotent stem cells commitment to a lung epithelial cell lineage.
	C. Lee	Pro-inflammatory cytokines and chemokines induced by mechanical stretch of myometrial cells can promote neutrophil infiltration by enhanced adhesion and transendothelial migration.
	M. Kwan	Dynamic changes in maternal decidual leukocyte populations across first and second trimesters of human gestation.
2:45 – 3:00	**Break**	
3:00 – 3:45	**EDRG Platform Oral Presentation** (MacLeod Auditorium)	
	C. Dong	Role of the intestinal epithelial insulin-like growth factor-1 receptor in glucagon-like peptide-2-mediated enhancement of intesitnal barrier function.
	Y. A. Chiang	P21-activated protein kinase 1 (Pak1) mediates the crosstalk between insulin and β-catenin on proglucagon gene expression and its ablation affects glucose homeostasis.
	A. N.-Armavil	Effects of insulin resistance on leptin modulation of hypothalamic neurons.
3:45 – 4:00	**Break**	
4:00 – 6:00	**Awards Ceremony and Reception** (MacLeod Auditorium - Foyer)	

32nd Frontiers in Physiology Floor Plan



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Keynote Lecture



"Application of Optogenetics"

Karl Deisseroth, M.D., Ph.D.

A native of Boston, Dr. Deisseroth received his bachelor's degree from Harvard in 1992, his PhD from Stanford in 1998, and his MD from Stanford in 2000. He completed postdoctoral training, medical internship, and adult psychiatry residency at Stanford, and he was board-certified by the American Board of Psychiatry and Neurology. He is a faculty member in the Bioengineering and Psychiatry Departments at Stanford, and continues as a practicing inpatient and outpatient psychiatrist at Stanford, employing medications and interventional brain stimulation techniques (VNS, TMS and others) to treat patients with psychiatric disease. In addition he serves as Director of Undergraduate Education in Bioengineering at Stanford, teaches yearly courses in both the graduate and undergraduate curricula, and provides education and training in optogenetics as well as freely distributing and supporting tools and expertise to thousands of scientists worldwide. Among other awards, for developing and applying optogenetics, Deisseroth has received the NIH Director's Pioneer Award and the McKnight Foundation Techological Innovations Award, and was the sole recipient of the 2010 Koetser Prize, the 2010 Nakasone Prize, and the 2011 Alden Spencer Prize. In 2010 he was elected to the Institute of Medicine.

MESSAGE FROM THE CO-CHAIRS OF FRONTIERS IN PHYSIOLOGY

On behalf of the Graduate Association of Students in Physiology (GASP), it is our great pleasure to welcome everyone to the 32nd Annual Frontiers in Physiology (FIP). FIP is a symposium that showcases and celebrates the cutting-edge research across all four platforms within the Department of Physiology. It is also a venue for exchange of scientific ideas amongst students and faculty members within the University of Toronto and its affiliated teaching hospitals and research institutions.

This year we are honored to welcome **Dr. Karl Deisseroth**. Dr. Deisseroth is a professor of Bioengineering and Psychiatry at Stanford University and the Howard Hughes Medical Institute. Dr. Karl Deisseroth led the development and application of optogenetics: the integration of genetics and optics to achieve gain- or loss-of-function of well-defined events (such as action potential patterns) in defined cell types within intact biological systems. We are delighted to have Dr. Deisseroth deliver his keynote lecture this morning, titled *"Application of Optogenetics"*.

The events of today were made largely possible by the combined efforts of a fantastic team – the Graduate Association for Students in Physiology (GASP). GASP could not have put together this research symposium without the continuing support of the Department of Physiology: the chair of the department, **Dr. Stephen Matthews**; the Graduate Coordinators, **Drs. Denise Belsham** and **Martin Wojtowicz**; and the departmental administrative staff. We would also like to thank all the faculty and postdoctoral fellows who volunteered their time as judges. Finally, many thanks extended to our institutional and commercial sponsors for their financial support: the Department of Physiology research platforms, University of Toronto Faculty of Medicine, University of Toronto Neuroscience Program, Graduate Students' Union, UTPoster.com, Cedarlane, Bio-Rad, Roche, VWR, Pall, Fisher and PeproTech.

Thank you once again to all who made this day possible, as well the many participants of FIP. We hope that all of you have a wonderful experience.



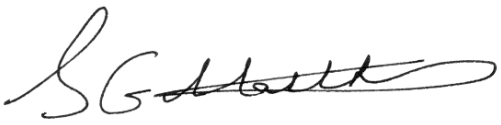
Majid Iqbal and Lemieux Luu
Co-Vice-Presidents of Graduate Association of Students in Physiology
Co-Chairs of Frontiers in Physiology

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 32 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.

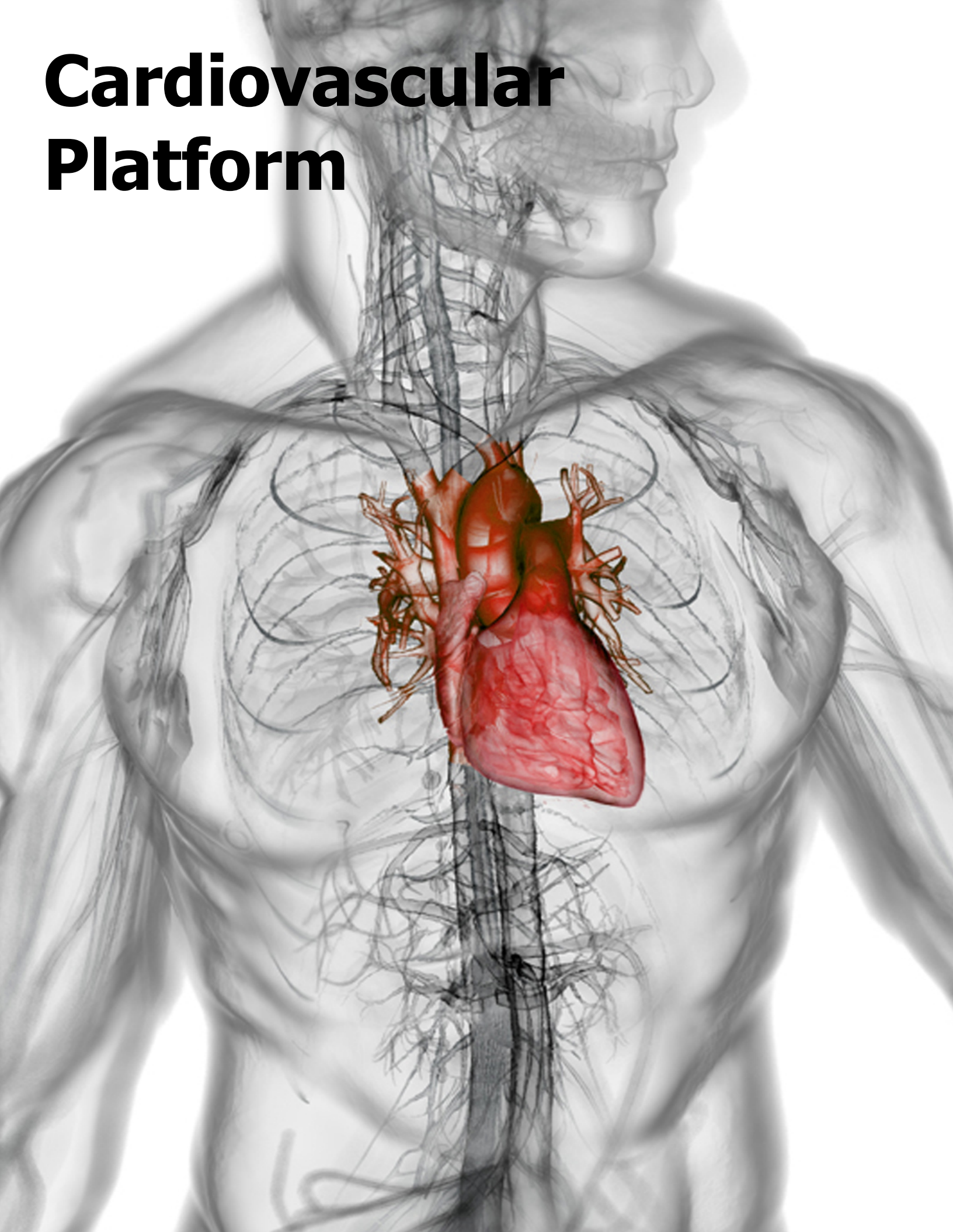
Special thanks and recognition is due today to **Mr. Majid Iqbal** and **Mr. Lemieux Luu**, FIP co-Chairs and Vice-Presidents of the Graduate Association of Students in Physiology (GASP). Majid and Lemieux's team have done an outstanding job and have generated an excellent program. Please join me in also thanking **Mr. Sean McFadden**, 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
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Cardiovascular Platform



Cardio: Orals

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

M. Sauvé[1] and S.S. Bolz[1].

Department of Physiology[1], Faculty of Medicine, University of Toronto, Ontario*, Canada.*

Diabetes is a primary risk factor for cardiovascular diseases, which represent the main cause of associated mortality. Dysfunction initiated by diabetes of pre-capillary resistance arteries, which regulate tissue perfusion and systemic blood pressure, has the potential to compromise blood flow and thus, organ integrity. VSMCs posses the intrinsic property to convert a mechanical stimulus (e.g., transmural pressure) into biochemical signals to regulate contractility. This *myogenic response* (MR) is the primary molecular mechanism regulating resistance artery tone. Our previous research investigated the role of the sphingosine-1-phosphate (S1P) signaling pathway (mediated via sphingosine kinase 1 (Sphk1) and the $S1P_2$ receptor) in regulation of MR. We demonstrated that tumour necrosis factor alpha (TNFα) augments resistance artery MRs via an S1P-dependent mechanism in the inflammatory disease conditions heart failure, subarachnoid hemorrhage, and vascular-based sudden hearing loss. The mechanistic consistency across these disease models suggests the presence of a fundamental mechanism common to inflammatory conditions. As diabetes also associates with elevated TNFα levels, *we propose that activation of S1P signaling in VSMCs by TNFα significantly contributes to diabetic microvascular dysfunction*. Diabetes is induced in mice by high fat diet/streptozotocin protocol. MR is augmented in isolated mesenteric and posterior cerebral arteries (PCAs) from diabetic mice. The maximal passive diameter is smaller in mesenteric arteries and PCAs from diabetic mice, suggesting a structural (remodeling) component. Acute treatment with etanercept therapy (TNFα antagonist) attenuates the diabetes-induced augmentation of MR in mesenteric arteries. Data obtained from $Sphk1^{-/-}$ mice and acute administration of the $S1P_2R$ receptor antagonist JTE013 outline a normalization of increased MRs in diabetic mice, demonstrating that diabetes-induced enhancement of MR in mesenteric arteries is S1P signalling-dependent. Our data suggests that TNFα regulates changes in MR in diabetes through the activation of microvascular S1P signaling, thereby identifying both pathways as therapeutic targets to mitigate microvascular dysfunction in diabetes.

ALPHA 1-ANTITRYPSIN IMPROVES LUNG FUNCTION IN A RAT LUNG TRANSPLANTATION MODEL

Wenxi Gao[1,3], Manyin Chen[3], Jinbo Zhao[3], Marcelo Cypel[2,3] , Shaf Keshavjee[2,3] and Mingyao Liu[1,2,3].

Department of Physiology[1], and Surgery[2], University of Toronto, Ontario, Canada. Latner Thoracic Surgery Research Laboratories, University Health Network[3], Toronto, Ontario, Canada.

Ischemia-reperfusion injury (IRI) is a severe complication in lung transplantation. Alpha 1-antitrypsin (A1AT) is a protease inhibitor, which is currently in clinical use for the treatment of A1AT deficiency emphysema. A1AT has been shown to have the potential to reduce IRI through its anti-inflammatory and anti-apoptotic effects. We hypothesized that A1AT improves lung function in IRI in lung transplantation. A rat single lung transplantation model was used. A1AT or placebo was administered intratracheally to donor lungs prior to harvest and intravenously to recipients prior to reperfusion (20 mg/rat/route). Rats underwent left lung transplantation after 18 h cold ischemic time followed by 2 h of reperfusion; during which, the recipient lung was ventilated with a Harvard ventilator for life support, while the lung graft was ventilated independently with flexiVent™, which assessed lung function mechanics (respiratory system resistance (Rrs), elastance (E) and peak airway pressure) every 30 min. After reperfusion, arterial blood gases from the transplanted lung were measured. Lung function mechanics of A1AT treated rats were improved at all time points. At 60 min of

reperfusion, A1AT group showed significant improvement in Rrs (1.9±0.4 vs. 2.9±0.9, $p<0.05$) and E (25.4±6.2 vs. 36.2±11.4, $p<0.05$) compared to the control group. Donor lung oxygenation in the A1AT group was significantly higher than that of the control group (258±82 vs. 105±26 mmHg, $p<0.05$). This separate ventilation model allows us to continuously measure independent function of the transplanted lung in order to test the efficacy of therapies on early post transplant lung function. We have shown that A1AT has protective effects against IRI in lung transplantation leading to significantly improved oxygenation and lung mechanics. Further studies are being undertaken to elucidate the mechanism by which A1AT ameliorates IRI. There is potential for future application of A1AT in the treatment of IRI in lung transplant patients.

INTRACELLUAR TRAFFICKING OF REGULATOR OF G-PROTEIN SIGNALING 4 (RGS4) REQUIRES PALMITOYLATION AND RAB FAMILY FUNCTION

G. Bastin[1,2], and S. Heximer[1,3].

[1]Department of Physiology, University of Toronto, Ontario, Canada, [2]Université des Sciences et Technologies de Lille, France, [3]Heart and Stroke Richard Lewar Centre for Excellence in Cardiovascular Research.

RGS proteins are potent inhibitors of heterotrimeric G-protein signaling. RGS4 attenuates G-protein activity in several tissues such that loss of its function may contribute to bradycardia, diabetic cardiomyopathy, breast cancer cell invasion, insulin resistance, and schizophrenia. RGS4 has been localized to both plasma membrane and intracellular membrane pools, however, the nature of intracellular trafficking remains to be been elucidated. G-protein inhibition requires the presence of RGS4 at the plasma membrane. The Rab family of proteins is known to facilitate intracellular trafficking of proteins through various vesicular and endosomal compartments via both retrograde and anterograde pathways. We used live cell imaging confocal microscopy to uncover a marked colocalization between RGS4 and Rab11 on intracellular endosomes. We asked whether Rab11, a key element in endosomal protein recycling and exocytosis may be important for plasma membrane targeting of RGS4. Indeed, when co-expressed dominant negative Rab11, RGS4 showed reduced plasma membrane targeting and an impaired ability to inhibit M1 muscarinic receptor mediated signaling. In parallel, we showed that RGS4 plasma membrane levels could also be regulated at the level of the endocytic machinery. Specifically, overexpression of Rab5a, the Rab family member required for clathrin-mediated endocytosis, decreased the presence of RGS4 at the plasma membrane and impaired its M1 muscarinic receptor inhibitory function. Interestingly, addition of 2-BP, an inhibitor of palmitoylation, prevented targeting of RGS4 to endosome compartments and the plasma membrane. Mutations of RGS4 Cysteine2 and Cysteine12, two putative palmitoylation sites, respectively disrupted localization to endosomal structures and plasma membrane targeting, these mutations lowered RGS4 inhibition of Gq mediated signaling. Taken together, these data provide the first evidence of Rab-dependent intracellular trafficking of RGS4 with the participation of its N-terminal palmitoylation sites and provides the tools for identifying new strategies aimed at increasing the function of RGS4 in living cells.

Cardio: Posters

C1. HUMAN NEUTROPHIL PEPTIDES INDICE ENDOTHELIAL-LEUKOCYTE INTERACTION, ACCLERATE FOAM CELL FORMATION, AND PLATELET AGGREGATION

A.A. Luo[1-4], K.L. Quinn[1-4], M. Henriques[1-4], A. Tabuchi[1,2,5], K.C. Cheng[1,6], W.E. Cheng[1-4], S. Tole[7], H. Yu[1-4], , E. Charbonney[1,5], E. Tullis[1,4], L.A. Robinson[7], H. Ni[1,2], W.M. Kuebler[1,2,5], A.S. Slutsky[1,4], H. Zhang[1-4].
The Keenan Research Centre in the Li Ka Shing Knowledge Institute of St. Michael's Hospital[1], Toronto, ON, Canada; Cardiovascular Collaborative Program in the Department of Physiology[2], Department of Anaesthesia3, Interdepartmental Division of Critical Care Medicine[4], Department of Surgery[5], University of Toronto, Toronto, ON, Canada; Department of Critical Care Medicine[6], Chi-Mei Medical Center, Tainan, Taiwan; Division of Nephrology[7], The Hospital for Sick Children, Toronto, Ontario, Canada.

Recent research has instigated a shift in the atherosclerosis paradigm from a lipid retention disease to a cascade of complex inflammatory events involving leukocyte recruitment, adhesion and migration, foam cell formation, and platelet aggregation. Polymorphonuclear neutrophils (PMN) are one of the main leukocytes recruited at the site of atherosclerotic lesions and their presence during plaque rupture and thrombi in patients with acute coronary syndrome raise numerous questions regarding their atherogenesis role. Human neutrophil peptides (HNP), constituting up to 50% of the total protein content in the azurophilic granules and are released upon PMN activation, has been found in the lesion area of coronary artery diseases. We hypothesized that HNP play an important role in PMN-mediated inflammatory cardiovascular responses in atherosclerosis. Human coronary artery endothelial cells, macrophages and platelets were stimulated for 4-hrs, 8-hrs and 2-mins, respectively, with HNP. Upon stimulation at 10μg/mL of HNP, human coronary artery endothelial cells showed increased monocyte adhesion and transmigration, induction of oxidative stress that accelerated foam cell formation in human macrophages, and activation and aggregation of human platelets. In conjunction, stimulation of superoxide dismutase or anti-CD36 antibody reduced the foam cell formation and cholesterol efflux. Lung endothelial cells isolated from wild type mice showed increased VCAM-1 and ICAM-1 expression upon HNP stimulation in comparison to LRP8 knockout mice, the only LRP phenotype expressed in platelets. The role of HNP was also studied *in vivo* in transgenic atherosclerotic mouse model. Mice deficient in double genes of low-density lipoprotein receptor (LDLR) and LDL receptor-related protein (LRP), and mice deficient in single gene of LRP8, show reduced leukocyte rolling and decreased platelet aggregation and thrombus formation in response to HNP stimulation. Our data suggests that HNP exert pro-atherosclerotic properties that appear to be mediated through LRP8 signaling pathways, suggesting an important role for HNP in the development of inflammatory cardiovascular diseases.

C2. CHRONIC SYSTEMIC INSULIN TREATMENT ATTENUATES ATHEROSCLEROTIC LESION DEVELOPMENT IN CHOLESTEROL-FED APOE -/- MICE

S. Chiang[1], J. Britto[1], A. Giacca[1].
Departments of Physiology[1], University of Toronto, Ontario, Canada.

Diabetes mellitus and metabolic syndrome, which are states of insulin resistance, are both well-known to increase the risk of atherosclerosis. However, it is still debated whether this increased risk is due to excess or lack of insulin action. Here, we present evidence that insulin is anti-atherogenic and decreases atherosclerosis independent of its effects on glycemia. ApoE -/- mice were fed a 1.25% (w/w) high cholesterol diet for 1 week pre-treatment then implanted with either control pellets or insulin pellets (0.05U/day which achieves a mild 4-fold elevation of circulating insulin without significantly lowering plasma glucose) subcutaneously for 12 weeks thereafter. At the end of the treatment, the mice were perfused and the aortas were harvested. Using en face Oil-Red O staining, the atherosclerotic plaque in the descending aorta was evaluated by measuring lesion area over total area. Insulin-treated mice (n=7) showed a significant decrease in atherosclerotic plaque (9.0 ± 2%) compared to control (n=11; 17.6 ± 2.5%). There was no significant difference in glycemia between the two groups. Based on our results, insulin is anti-atherogenic in the context of euglycemia.

C3. VALIDATION AND MECHANISMS OF NOVEL COMPOUNDS MODULATING ANGIOGENESIS.

J. Tat[1,3], Y. Wang[3], M.Y. Liu[1], X.Y. Wen[2,3].
Departments of Physiology[1] and Medicine[2], Faculty of Medicine, University of Toronto, Li Ka Shing Knowledge Institute[3], St. Michael's Hospital, Toronto, Ontario, Canada.

Abnormal angiogenesis is featured in over 70 health conditions and is a critical component of tumour growth and metastasis. Discovering novel therapeutic compounds that stimulate or abrogate this process can lead to the development of new treatments for cancer and tissue regeneration. The zebrafish has emerged as an important model for studying vascular development and angiogenesis. Advantages over other models include size, fecundity, rapid embryonic development and optical clarity of the embryos. The goal of my project is to validate the potential therapeutic efficacy of compounds identified from an angiogenesis chemical genetic screen and investigate their molecular mechanisms. Two compounds are being studied; the pro-angiogenic compound retinoic acid p-hydroxyanilide (4HPR) and the angiogenic inhibitor indirubin-3-oxime (IO). In a developmental angiogenesis assay performed on transgenic zebrafish embryos, 4HPR promoted vessel development beyond normal boundaries, to the tail tip. However, a physiological angiogenesis assay resulted in significant inhibitory effects on larval and adult fin regeneration. IHC staining reveals that somite extension to the tail tip persists with the combined treatment of 4HPR and the VEGF inhibitor SU5416. These findings suggest that 4HPR promotes somitogenesis during development (to which proangiogenesis is a secondary effect), while in the adult it functions as an angiogenic inhibitor. IO is an angiogenesis inhibitor that decreases the number of vessels in developing zebrafish while abrogating fin regeneration in larval and adult zebrafish. IO also inhibits tumour angiogenesis in a zebrafish embryo xenograft model. Cell counting in transgenic zebrafish with nuclear-specific labeling suggests that endothelial cell migration is specifically being affected. Combined dosing with the notch inhibitor DAPT appears to rescue the IO phenotype, demonstrating that IO action is possibly through enhanced notch

signaling. Understanding the mechanisms of action is the next step in developing these two compounds as new therapies for conditions with defective and excessive angiogenesis.

C4. ROLE OF SLC6A14 AS A MODIFIER OF CYSTIC FIBROSIS PHENOTYPE

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

Cystic Fibrosis (CF) is considered to be a single gene disorder, most commonly the result of a F508del mutation in the CFTR gene. Patients homozygous for F508del mutation show a considerable variation in their phenotype. Consistent with this observation, recent genome wide association studies indicate the role of modifier genes which can modify the disease phenotype. A Single Nucleotide Polymorphism in the putative promoter region of SLC6A14 gene showed the highest significance ($p=1.28x10^{-12}$). SLC6A14 is a cationic/neutral amino acid transporter, which transports $2Na^+$, 1 Cl^- and a basic/neutral amino acid into the cell from the apical surface of intestinal/bronchial epithelium. We hypothesize that the expression of SLC6A14 is protective against meconium ileus. To test this hypothesis we first performed mRNA expression analysis in the ileum and other mouse tissue samples. Preliminary results in WT mice show the highest expression in lung and colon, which are CF affected tissues. A construct of SLC6A14 with a FLAG tag was overexpressed in BHK cells, we see that the protein is both core and complex glycosylated, like CFTR. CFTR is a chloride channel and is also functionally expressed at the apical surface of epithelial cells. Our preliminary results with a halide flux assay using the chloride sensitive flurophore SPQ, show that the CFTR function is inhibited upon inhibition of SLC6A14 in cells coexpressing SLC6A14 and wild type CFTR or F508del CFTR. This suggests a possible interaction of SLC6A14 with CFTR. Also, our preliminary results in the 3 dimensional epithelial cultures forming cysts, show that the size of the cyst increases on CFTR stimulation, and a SCL6A14 inhibitor (α-methyltyrptophan) prevents this increase. These results show that SLC6A14 affects CFTR function thereby modifying CF phenotype, and serves as a potential prognostic genetic marker and drug target for CF patients. (Studies supported by CFC, CIHR, UofT).

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

J.T. Kroetsch and S. Bolz.
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The myogenic response (MR), i.e., the intrinsic property of resistance arteries (RA) to adjust resistance to changes in perfusion pressure, is the primary mechanism underlying the regulation of peripheral resistance. We demonstrated that a TNFα-dependent upregulation of MRs hallmarks several diseases with a systemic inflammatory component (e.g., heart failure (HF) and subarachnoidal hemorrhage). Based on these findings, we hypothesize i) that TNFα is a mechanosensitive physiological regulator of MRs in microvascular smooth muscle cells, ii) that microvascular TNFα signaling is upregulated in HF and (iii) that microvascular TNFα signaling is a therapeutic target to normalize MRs and hence, peripheral resistance in HF. Pressure-induced MRs (20-100mmHg in 20mmHg increments) and MLC_{20}-phosphorylation in C57/BL6 mouse cremaster muscle RAs were significantly reduced following treatment with the TNFα scavenger etanercept (ETAN). ETAN also reduced MRs in Syrian hamster gracilis muscle RAs. ETAN failed to inhibit MRs in TNF$\alpha^{-/-}$ RAs and boiling of ETAN abolished its inhibitory effect on MRs in hamster RA. ETAN did not alter responses to phenylephrine, norepinephrine, and acetylcholine. In HF, induced by ligation of the left anterior descending (LAD) coronary artery, cremaster muscle RA displayed significantly enhanced MRs at 6-8wks post-ligation (compared to sham-operated mice) in C57/BL6 wild-type but not in TNF$\alpha^{-/-}$ mice. Enhanced MRs in wild-type RAs were significantly reduced following acute in-bath treatment with ETAN. However, systemic treatment of LAD-ligated wild-type mice for 6 weeks with ETAN failed to reduce the HF-induced increase in MRs. Our data suggest that TNFα is an essential component of the mechanosensitive cascade that regulates MRs in skeletal muscle RA under physiological and pathophysiological conditions. The apparent compensation of long-term systemic TNFα sequestration supports the concept of the MR as a highly redundant mechanism and has important implications for therapeutic strategies.

C6. PROTEOMIC CHARACTERIZATION OF THE CARDIAC FIBROBLAST EXOSOME PROTEOME

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Exosomes are secreted vesicles of diameters ranging from 1 – 100 nm. These microvesicles are secreted into the extracellular space and used for: transport of waste metabolites, signal transduction, and transfer of genetic material to target cells. Proteomic analyses of exosomes of other cell types and bodily fluids have shown its potential in biomarker discovery. In the heart, current research has been limited to the role of exosomes in stress responses of cardiomyocytes. With the cardiac fibroblast (CF) being the largest cell population in the heart, by number, understanding its exosomal profile will provide insight on intercellular communication in the heart. Identification of the murine CF exosome proteome will provide insight on this understudied secretory pathway in the heart. Cultured neonatal CF were used as a source of cardiac exosomes. Conditioned media from these cultures was collected after 24-36 hr. Media was cleared of cell debris and larger microvesicles by differential centrifugation, concentrated then ultracentrifuged at 110 000 × g to pellet exosomes. Purified exosomes were characterized by electron microscopy and western blot, and prepared for proteomic analysis via urea solubilization and tryptic peptide digestion. Preliminary analysis on a 1D column setup in-line with an LTQ Orbitrap mass spectrometer identified an average of 239 ± 139 proteins per technical run over four biological replicates. Known exosomal markers such as CD81, HSP70, and the 14-3-3 family of signal transduction proteins were identified in the MS analysis. Ongoing work is focused on continued optimization of exosome isolation approaches using sucrose cushions and gradients, comparison to human adult cardiac fibroblast exosome, and profiling the cardiomyocyte exosome. Together, these studies will provide valuable insight into the role of the exosome in the heart.

C7. THE SEX-SPECIFIC INFLUENCE OF ADIPOSITY ON BLOOD PRESSURE DURING PHYSICAL AND MENTAL CHALLENGES IN ADOLESCENCE

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Greater blood pressure (BP) reactivity and slower BP recovery from physical and mental challenges predict future hypertension. BP and the prevalence of hypertension are higher in males than females throughout their reproductive age and excess body-fat is a major risk factor for hypertension in both males and females. We investigated whether BP reactivity and recovery from physical and mental challenges differ between adolescent males and females, and whether these hemodynamic parameters are associated with resting BP and are influenced by excess body-fat in a sex-specific manner. In 285 male and 311 female adolescents (age 12-18 years), we quantified visceral fat (VF) with magnetic resonance imaging and total body fat (TBF) with bioimpedance and measured systolic BP (SBP) beat-by-beat during a 52-minute protocol, which included posture and math-stress tests. Our results showed that males compared with females had enhanced SBP reactivity but equivalent SBP recovery from both challenges. In both sexes, greater reactivity and slower recovery from either challenge correlated closely with resting SBP, explaining up to 34% of their variance. In males, VF independently of TBF was associated with greater SBP reactivity to standing, whereas in females, TBF independently of VF was positively associated with slower SBP recovery from standing. These results suggest sex differences exist not only in BP adaptation to daily activities, but also in the way visceral and non-visceral body fat influence this BP adaptation. Overall, these results suggest the need for sex-specific prevention and treatment of hypertension.

C8. CHARACTERIZATION OF SLC6A14 IN HUMAN AIRWAY EPITHELIA: A POSSSIBLE ROLE IN AIRWAY FLUID HOMEOSTASIS AND CYSTIC FIBROSIS

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Cystic Fibrosis (CF) is caused by defects in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR). The initiating lesion in CF is a decrease in airway surface liquid (ASL) height causing poor mucociliary clearance and invasion of pathogens. This stems from a dysregulation of ion transport across the apical surface of respiratory epithelium mediated primarily by CFTR-dependant Cl^- secretion and Epithelial Na^+ Channel (ENaC) – mediated Na^+ absorption. Dysregulation of ASL height occurs when CFTR is absent and the normal chemical and electrical gradients are disturbed causing insufficient water secretion (as water follows Cl^- and Na^+ passively across the membrane). CFTR mutations alone, however, do not explain the heterogeneity seen in the CF phenotype which has spurred on the search for modifier genes. A recent Genome-Wide Association Study (GWAS) in CF has identified SLC6A14 as being strongly associated with an increased risk of Meconium Ileus in CF (a marker of a strong genetic component to the disease) as well as lung disease severity. SLC6A14 is an electrogenic NaCl-dependent broad-specificity transporter of neutral and cationic amino acids and is found on the luminal surface of many bodily tissues including the lung and colon. I hypothesize that altered expression of SLC6A14 therefore may interfere with the homeostasis of ASL height by altering sensitive electrical and chemical gradients. I will characterize SLC6A14 in the human bronchial cell lines 16HBE14o- (immortalized healthy bronchial epithelia) and CFBE41o- (HBE expressing dF508/dF508 CFTR) as well as primary cultures from donors and explants (normal and CF lungs) from the University of Iowa. These data will include protein expression through Western blot, and functional expression in Ussing chambers (transepithelial voltage (Vte), resistance (R), and equivalent short-circuit current (Isc) recordings) and [3H]-L-arginine uptake experiments. Western blot data indicate that SLC6A14 is present in HBE and CFBE cell lines and that it is detectable in primary cultures of both normal and CF lungs. In normal and CF primary cultures, SLC6A14 expression was positively correlated with Ussing chamber responses to L-arginine stimulation. This indicates that varying genetic expression of SLC6A14 may influence the electrical environment on the surface of the lung therefore also affecting fluid homeostasis and CF lung disease. Further studies will be directed at identifying any functional interaction between SLC6A14 and CFTR in the Ussing chamber and [3H]-L-arginine uptake experiments using cell lines and primary cultures, as well as ASL height measurements using line-scanning confocal microscopy in normal and CF cultures.

C9. ELUCIDATING THE MECHANISM OF ACTION OF THE GLP-1 METABOLITE, GLP-1(9-36), IN THE CARDIOVASCULAR SYSTEM

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The incretin hormone, glucagon-like peptide-1 (GLP-1) is a gut-peptide secreted from intestinal L-cells in response to oral nutrient ingestion. The majority of circulating GLP-1 is the 30 amino-acid GLP-1(7-36)amide which acts on a G-protein coupled receptor (GLP-1R) on pancreatic beta-cells to stimulate insulin secretion and regulate glucose production post-prandially. GLP-1(7-36) is however short-lived with a half-life averaging 1-2 min, and is rapidly degraded by enzyme dipeptidyl-peptidase-4 to the longer-acting GLP-1(9-36)amide, an N-terminal derived peptide. GLP-1(9-36) is devoid of insulinotropic and glucoregulatory effect but has shown vasodilatory and cardioprotective actions when applied post-ischemia in isolated hearts of both wild-type (WT) and GLP-1R knockout (*Glp-1r*$^{-/-}$) mice. We hypothesized that GLP-1(9-36) exerts cardiovascular actions independent of the GLP-1R receptor, through a yet unidentified signaling mechanism. Recent reports indicate that a nonapeptide (GLP-1(28-36)), produced through neutral endopeptidase (NEP) mediated cleavage of GLP-1(9-36)amide, targets to mitochondria of isolated mouse hepatocytes to exert insulin-like actions by modulating oxidative-phosphorylation. We aimed to determine whether the GLP-1R-

independent cardioprotective actions observed with GLP-1(9-36) were actually being modulated by breakdown to GLP-1(28-36). We used the *ex vivo* Langendorff model of ischemia/reperfusion (I/R) injury to characterize the cardioprotective actions of GLP-1(9-36) and (28-36) versus GLP-1(7-36) as positive control and 'untreated' as negative control. A 20 min pre-treatment of isolated, WT, 10-12 weeks old male mice with GLP-1(9-36) or (28-36) prior to ischemia, significantly improved recovery of left ventricular developed pressure at the end of 40 min reperfusion, as compared to untreated controls ($P<0.05$). The recovery observed with GLP-1(9-36) and (28-36) were comparable to parent peptide GLP-1(7-36). We also measured lactate dehydrogenase (LDH) release as a measure of myocyte cell death following I/R injury. Pre-treatment with GLP-1(9-36), (28-36) and parent (7-36) all significantly decreased LDH release in coronary effluents during reperfusion period as compared to untreated controls ($P<0.05$). Our data suggests that both GLP-1(9-36) and (28-36) are equally cardioprotective in I/R settings. To determine whether these effects are mediated by breakdown of (9-36) into (28-36), future experiments will be directed at testing the *ex vivo* cardioprotective actions of GLP-1(9-36) in the presence of NEP inhibitor, candoxatril.

C10. ROLE OF SPHINGOSINE-1-PHOSPHATE SIGNALLING IN HUMAN RESISTANCE ARTERY FUNCTION IN CLINICAL HEART FAILURE

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Resistance arteries (RAs) regulate tissue perfusion, total peripheral resistance (TPR) and systemic blood pressure. As the primary mechanism, intrinsic pressure-dependent *myogenic responses* continuously adapt RA resistance to changes in transmural pressure. On the molecular level, Sphingosine-1-phosphate (S1P) signalling is one key regulator of myogenic responsiveness under physiological and pathophysiological conditions. Mice with heart failure (HF) display enhanced myogenic responsiveness that results from a TNFα-induced *enhancement* of microvascular S1P signalling. This molecular mechanism underlies the adaptive elevation of TPR to stabilize blood pressure and organ perfusion in early stage HF. Although initially beneficial, the TPR increase amplifies cardiac workload and exacerbates late stage HF, making the TNFα-dependent upregulation of S1P signalling a novel target for the treatment of heart failure. To translate this mechanism to *human* RAs (hRA), we hypothesize that S1P signalling is critical for the regulation of hRA myogenic responsiveness and that hRA from HF patients show the same functional and molecular signatures as RAs from HF mice (i.e., TNFα-induced upregulation of S1P signalling leading to enhanced myogenic responsiveness). Medical chart review will provide clinical data for grouped analyses (e.g. HF stage, medications). Expression of S1P signalling components will be assessed by qRT-PCR, Western blot and immunostaining in hRA from patients without and at different stages of HF. Vascular reactivity and the functional role of S1P signalling for the regulation of hRA myogenic responsiveness will be determined using perfusion myography combined with chemical or genetic strategies to inhibit selected S1P signalling components (sphingosine kinase 1, S1P2 receptor). Recent data from hRA demonstrating dose-dependent constriction to phenylephrine and S1P, dilations to acetylcholine, pressure-induced myogenic responses and their augmentation by exogenous S1P successfully translate key findings from rodents to humans. This suggests a role for S1P signalling in hRA and identifies this signalling pathway as a potential therapeutic target in hRA.

C11. GENERATION AND CHARACTERIZATION OF ERP44 (TXND4) KNOCKOUT/KNOCKIN MICE TO STUDY THE ROLE OF ERP44 IN CA^{2+} SIGNALING AND ER STRESS IN THE HEART.

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Cardiovascular disease is the leading cause of suffering and death in Canada. 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 and cell types. 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), and by supporting disulfide bond formation. Dysregulation of Ca^{2+} cycling in myocytes will activate ER stress pathway and lead to cell death in cardiomyocytes. I hypothesize that ERp44 plays important roles in ER stress involved in heart disease by regulation of Ca^{2+} signaling and activation of the ER stress response signaling. To investigate the role of ERp44 in Ca^{2+} signaling and ER stress *in vivo*, we have generated an ERp44 knock-out/knock-in mouse model. The health and survival rate of wildtype, heterozygous, and ERp44 knockout mice has being assessed over time. Initial results indicate that there are severe phenotypic differences between the ERp44 knockouts compared to the wildtype mice. The ERp44 knockouts have low body weight, decreased activity or reluctance to move, and un-groomed hair. The survival rate of the ERp44 knockout is extremely low (<25%), with the majority of the ERp44 knockouts dying during early embryonic development. Wiltype and heterozygous embryos were Lacz stained at different developmental stages and showed a high expression of this gene in early embryonic stages and a decline of expression as the embryo age. Neonatal cardiomyocytes were also cultured and assessed for Ca^{2+} transients using the Ca^{2+}indicator, Fura-2 (1uM). Preliminary results illustrated that there is a higher Ca^{2+} release amplitude and lower frequency in the ERp44 knockout and heterozygous versus the wildtype neonatal cardiomyocytes. Therefore, my results suggest that ERp44 plays a significant role in early embryonic stages and that ERp44 may be involved in negative regulation of Ca^{2+} transient.

C12. IDENTIFICATION OF A NOVEL BACKGROUND SODIUM CURRENT CONTRIBUTING TO PACEMAKER GENERATION IN MOUSE ISOLATED SINO-ATRIAL NODE MYOCYTES.

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Pacemaker activity of the sino-atrial node (SAN) requires complex and dynamic ionic currents that are carried through diverse groups of ion channels. Unlike non-pacemaking cells of the right atrium, SAN cardiomyocytes requires background sodium current to establish the highly depolarized resting membrane potential necessary for pacemaking generation. While HCN has been considered as a major contributor to rhythmic activities, it does not fully explain the relatively more depolarized resting

membrane potential in SAN cells. Recent studies in the nervous system have identified NALCN (Na^+ leak current, non-selective) as a candidate to the background Na^+ conductance. Our recent studies in *Lymnaea stagnalis* showed that knockdown of a NALCN orthologue reduced neuronal background Na^+ current of a pacemaker cell and abolished/altered spontaneous activity due to hyperpolarization of resting membrane potential (Lu and Feng, PLoS One, 2011, 6(4):e18745). Tissue expression profile indicates NALCN gene is highly expressed in the heart. In this study, we hypothesizes that NALCN-like current is similarly involved in pacemaker generation in the SAN cardiomyocytes. To test this hypothesis, we carried out patch-clamp recordings and used pharmacological approach to isolate the NALCN-like current in isolated adul mouse SAN myocytes. Our data suggests that a NALCN-like Na^+ current contributes to the membrane potential of SAN myocytes. We further studied the properties of the channels using recombinant DNA approach. Our findings indicate that functional NALCN channels required coexpression of auxillary subunit in tsA201 cells. Taken together, our results provided the first identification of a novel current involved in pacemaker generation of SAN cardiomyocytes.

C13. MECHANISM OF ENDURANCE EXERCISE-INDUCED ATRIAL FIBRILLATION.

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Atrial fibrillation (AF), the most common supraventricular arrhythmia, can occur without any underlying cardiovascular disease (lone AF, LAF). Middle aged males who engage/have engaged in high intensity endurance exercise account for a vast proportion of LAF patients. The underlying mechanisms accounting for this association are currently unknown. We found large elevations of atrial pressure with exercise in mice, we thus hypothesized that increased AF with exercise training occurs due to atrial stretch and tissue damage ultimately resulting in remodeling of the atria to a more accommodating AF substrate. To investigate our hypothesis, we used a mouse model of endurance exercise. Mice were exercised twice daily for 1.5 hours in a round water bath (31–33˚C) for 4 weeks. A reduction in heart rate was observed as early as 2 weeks of training (501±64bpm in sedentary, 417±72bpm in exercised) and was maintained throughout the exercise protocol. After 4 weeks of training, a significant increase in contractility was observed in both the right ventricle (2055±268mmHg/s sedentary vs 3597±331mmHg/s trained) and left ventricle (7768±405mmHg/s sedentary vs 11408±542mmHg/s trained). AF susceptibility was assessed *in vivo* using an octapolar EP catheter guided into the right ventricle. Using a burst stimulation protocol (400ms duration, 20ms pulse interval) AF (>5s) was induced in 13/34 exercised mice compared to 1/27 sedentary controls. Susceptibility to AF was further characterized using optical mapping of Di-4-ANEPPS stained isolated atria preparations. AF was induced using a burst stimulation protocol (27-40ms pulses with 2ms decremental pulse interval to 20ms). Consistent with our *in vivo* studies, AF was inducible in 8/13 atria from exercised mice compared to 0/8 atria isolated from sedentary controls. Histological analysis for fibrosis using picrosirius red revealed increased peripheral and interstitial fibrosis in exercised atria but not in the ventricles (17.0±1.1% in exercised compared to 2.4±0.3% in sedentary control atria; 2.4±0.3% in exercised compared to 3.6±0.4% in sedentary control ventricles). Consistent with this observation, immunohistochemistry revealed macrophage infiltration in the atria but not ventricles, accompanied by up-regulation of TGFβ1 mRNA (2.8 fold) in atria from exercised mice. Our data shows endurance (swimming) exercise induces large elevations in atrial pressures, which in association with tissue inflammation and fibrotic collagen deposition leads to increased AF vulnerability. These studies will assist in understanding and preventing AF in athletes.

C14. MECHANISMS OF ATRIAL FIBRILLATION UNDER DIMINISHED CAV1.3 EXPRESSION

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Atrial fibrillation (AF) is the most common sustained supraventricular arrhythmia and is associated with increased propensity to stroke, impaired cardiac performance and cardiomyopathy. Interestingly, transgenic mice lacking the L-Type Ca^{2+} a1D subunit ($Ca_v1.3$) are found to be more susceptible to AF, however the underlying mechanisms for this observation are unknown. Characterization of $Ca_v1.3^{-/-}$ mice revealed the critical role of $Ca_v1.3$ in the pacemaker action potential (AP) of the heart. $Ca_v1.3^{-/-}$ atrial myocytes display diminished L-Type calcium current ($I_{Ca\text{-}L}$) density represented by a reduced $I_{Ca\text{-}L}$ peak density at +10 mV. Based on the evidence, we hypothesize that the increased vulnerability to AF observed in Cav1.3-/- mice is a result of decreased $I_{Ca\text{-}L}$ leading to a reduction in the action potential duration, and decreased atrial refractoriness. Using a rapid stimulation protocol we found 5/5 atria isolated from Cav1.3-/- mice exhibited AF with rapid stimulation, compared to 0/4 Cav1.3+/+ mice (27 stimuli at 40ms interval repeated with 2ms decrements to 20ms interval). Surprisingly, intracellular potential recordings revealed a prolongation of the action potential duration (APD) in Cav1.3-/- mice (APD_{30}: 7.3±0.8ms Cav1.3+/+, 10.4±0.7ms Cav1.3-/-, APD_{50}: 14.5±0.9ms Cav1.3+/+, 20.8±1.3ms Cav1.3-/-, APD_{90}: 46.9±4.1ms Cav1.3+/+, 64.0±6.3ms Cav1.3-/- n=8 for Cav1.3+/+ and n=6 for Cav1.3-/-; $p<0.05$). Surprisingly, Cav1.3-/- right and left atrial (RA and LA) appendages exhibited a 2-fold percent increase in atrial collagen fiber to total atrial tissue area (RA: 11.67±0.68 and LA: 11.13±0.93 Cav1.3+/+ and RA: 23.68±3.42 and LA: 21.84±1.11 for Cav1.3-/-, n=5 $P<0.0001$). Furthermore, optical mapping of isolated atria revealed a slowed conduction velocity (0.38±0.06m/s Cav1.3 +/+ n=3, 0.26±0.01m/s Cav1.3-/- n=2), and prolonged p-wave duration from ECG recordings of anesthetized mice (10.9±0.96ms Cav1.3+/+, 15.86±0.99ms Cav1.3-/- n=7 for Cav1.3+/+ and n=6 for Cav1.3-/-; $p<0.005$). Although Cav1.3-/- failed to display a decrease in atrial refractoriness, other substrates for AF are present (increased atrial fibrosis and delayed conduction velocity), and mechanisms for these observations are under further exploration.

C15. ROLE OF OXIDATIVE STRESS IN MEDIATING ELEVATED ATRIAL FIBRILLATION BY TUMOR NECROSIS FACTOR-ALPHA

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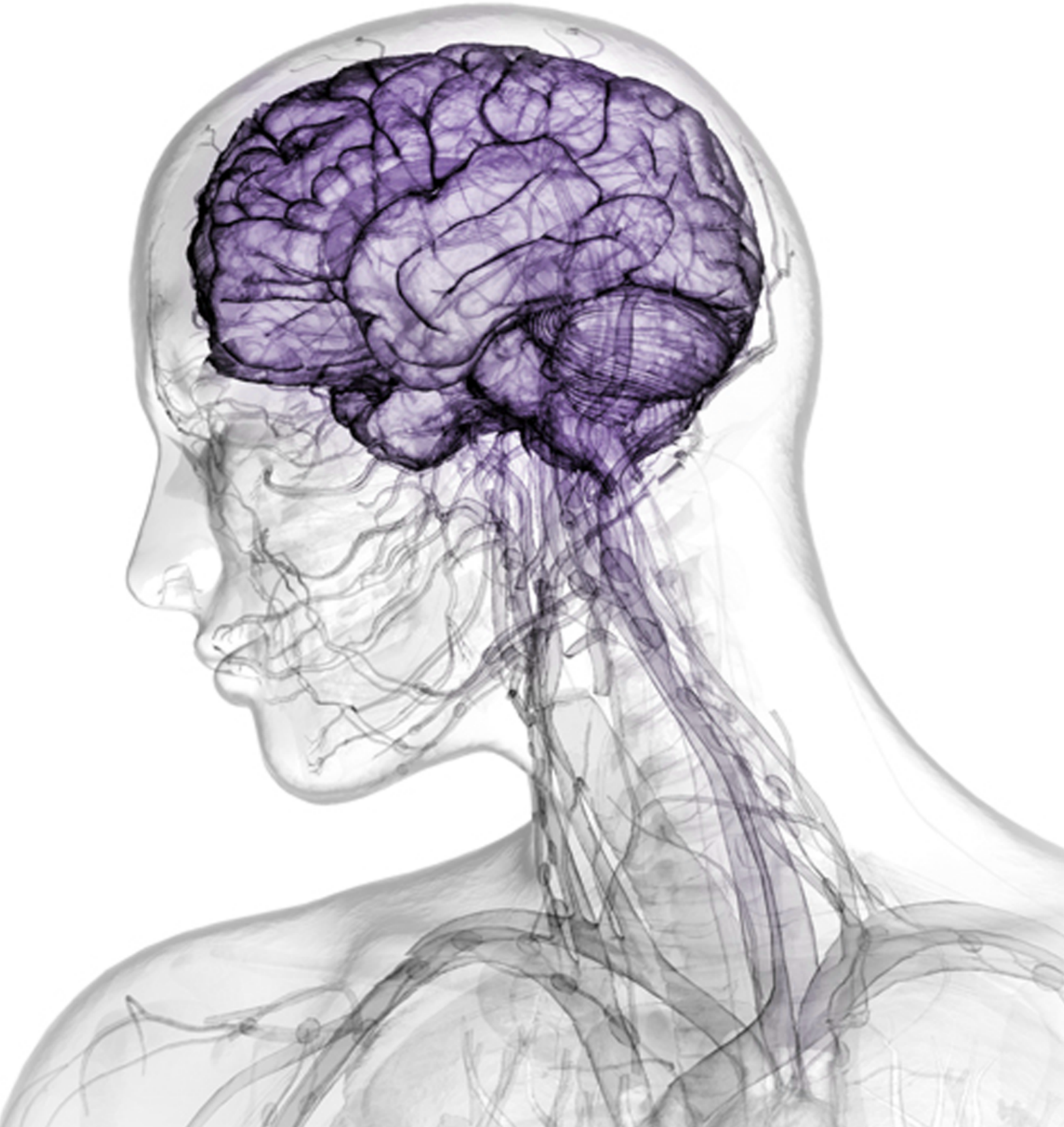
Atrial fibrillation (AF) is the most common arrhythmia encountered in clinical practice. The mechanisms underlying paroxysmal (non-sustained) AF are not completely understood. The incidence of AF strongly correlates with increase in inflammatory cytokines such as tumor necrosis factor- α (TNF- α), C-reactive protein and IL-6. Mice with chronic overexpression of TNF-α have show increased susceptibility to AF and increased sarcoplasmic reticulum (SR) Ca^{2+} leak. TNF- α is potent inducer of oxidative stress and AF is also associated with elevated oxidative stress. We have shown that acute exposure of atrial tissue to TNF- α increases susceptibility to AF. Hence we hypothesize that TNF-α alters Ca^{2+} homeostasis in atrial myocytes through elevated oxidative stress via an increase in SR spontaneous Ca^{2+} release (Ca^{2+} sparks), leading to delayed afterdepolorizations (DADs), premature atrial beats and triggered arrhythmias. Spontaneous Ca^{2+} activity was measured in electrically paced isolated mouse atrial cardiomyocytes loaded with Fluo-3-AM dye and treated with 20ng/ml TNF-α for one hour, using confocal microscopy. Isolated atrial myocytes treated with TNF-α showed increased frequency of Ca^{2+} sparks (5.6± 0.5 Sparks/S.(100μm), n=22) compared to control myocytes (2.7 ± 0.3 Sparks/S.(100μm), n=21, $P<0.01$), with increased incidence of DADs. The level of reactive oxygen species (ROS) measured in TNF-α treated isolated atrial myocytes (n=9) using the CM-H_2DCFDA dye showed 26% increase in ROS levels compared to control cells (n=9). In order to determine the source of ROS, TNF- α treated and control atrial myocytes were incubated with apocynin, NADPH oxidase inhibitor. Apocynin (100 μm), reduced the frequency of sparks in TNF- α treated cells (from 6.8 ± 0.6 to 4.0 ± 0.3 Sparks/S.(100μm), n=12, $P<0.01$), to the levels indistinguishable from untreated control cells (4.2 ± 0.5 Sparks/S.(100μm), n=10). Treatment with MitoTEMPO (25nM), a mitochondrial targeted superoxide scavenger, also caused a reduction in spark frequency in TNF- α treated cells from 7 ± 0.5 to 4.4 ± 0.3 Sparks/S.(100μm),($P<0.01$). These results suggest that TNF-α increases susceptibility to AF most likely through altered Ca^{2+} handling and increased spontaneous Ca^{2+} release due to elevated ROS.

C.16 DELTA-F508-CFTR'S FUNCTION AND EXPRESSION CAN BE PARTIALLY RESTORED THROUGH INHIBITION OF AMPK SIGNALING

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Sphingosine-1-phosphate (S1P) directs an array of biological processes. Its bioavailability is controlled by rheostat between sphingosine kinase 1 (Sphk1)-dependent generation and S1P phosphohydrolase 1 (SPP1)-dependent degradation. We have previously shown that S1P- import critically depends on the CFTR (cystic fibrosis transmembrane regulator) chloride channel, and that changes in its function regulate S1P signaling. We recently identified S1P as an upstream, negative regulator of CFTR's function, that mediates its effect through AMPK and phosphorylation. Here, we hypothesized that CFTR dysfunction, as it occurs Cystic Fibrosis (CF), may be related to an up-regulation of S1P/AMPK-signalling. Thus, inhibition of AMPK-signalling may restore CFTR function by increasing CFTR activity as well as its expression at the plasma membrane. We measured the CFTR channel conductance in BHK-dF508-CFTR stable cells, the most common mutation among CF patients, using the conventional iodide efflux technique. Its minimal cell-surface expression leads to abnormal conductance. Low-temperature rescue (27C, 24hrs) showed an increased conductance. Treatment with Compound C (1uM, 24hrs), an AMPK inhibitor, had similar effects to temperature rescue and augmented channel conductance. Specific inhibition of AMPK with siRNA (25nM, 48hrs) also enhanced channel conductance, an effect that was absent in the negative control pool. To confirm that expression of intact CFTR at the plasma membrane is crucial for S1P bioavailability, dF508-CFTR cells, which have reduced cell surface expression, have impaired FITC-S1P uptake as assessed by FACS analysis which was rescued by low temperature incubation. Finally, Compound C pre-treatment (20uM, 1hr) increased FITC-S1P uptake and cell surface expression of dF508-CFTR indicating that the restored channel function requires CFTR localization to the membrane. Our study shows that up-regulation of AMPK signaling leads to impaired CFTR function, resulting in reduced channel conductance and S1P-transport as well as decreased expression at the cell-surface. Inhibition of AMPK through either pharmacologic (e.g., Compound C) or genetic intervention (e.g., AMPK siRNA) successfully restores dF508-CFTR's function and expression promoting the concept that AMPK modulation may correct CFTR-related pathologies.

B.R.A.I.N. Platform



B.R.A.I.N.: Orals

COUPLING OF P2Y-MEDIATED STORE DEPLETION & CA^{2+} ENTRY (SOCE) TO SK4 CHANNELS AND MICROGLIA MIGRATION

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We recently published that SK3/KCa2.3 and SK4/KCa3.1 channels contribute to microglial activation and neurotoxicity via regulation of p38 MAPK activation; and were intrigued that two SK channels are involved in the same outcomes. Thus, we asked whether they are functionally coupled to different Ca^{2+} sources. Damaged neurons release ATP, a purinergic receptor ligand that can activate microglia and enhance chemotactic migration by raising intracellular Ca^{2+}. Purinergic responses can be mediated by ionotropic P2X or metabotropic P2Y receptors. Here, we stimulated MLS-9 cells (a microglia cell line that expresses both SK3 and SK4) with UTP to trigger P2Y-mediated responses; i.e., depletion of intracellular Ca^{2+} stores, and subsequent store operated Ca^{2+} entry (SOCE). In Fura-2 loaded cells, UTP evoked a rapid Ca^{2+} transient, followed by a second, more sustained rise due to SOCE. The second phase was decreased by the inhibitor, 2-APB, which we recently showed blocks Ca^{2+} release-activated Ca^{2+} (CRAC) channels in microglia. In whole cell recordings, UTP activated a robust current, which was blocked by the SK4 inhibitor, TRAM-34 (but not by the SK3 blocker, apamin), and was therefore, SK4/KCa3.1. SK4 activation by UTP was also inhibited by 2-APB, suggesting that SOCE is required for, and coupled to, SK4 channel activation. SK4 activity was apparently needed for Ca^{2+} entry and store re-filling; the SK4 blocker, TRAM-34, accelerated the decay in internal Ca^{2+} after UTP, and reduced the amplitude of a second Ca^{2+} release from stores evoked by the SERCA pump inhibitor, thapsigargin. In functional assays, SOCE and SK4 channels contributed to the migratory capability of primary microglia stimulated by UTP. Our data support a selective coupling of SK4 channels (but not SK3) to Ca^{2+} entry through SOCE/CRAC. Our current model is that SK4 activation maintains a hyperpolarized membrane potential that helps drive Ca^{2+} entry through CRAC, and facilitates store refilling.

PRESERVATION OF MECP2 IN CATECHOLAMINERGIC CELLS IS SUFFICIENT TO IMPROVE LIFE SPAN AND BEHAVIOURAL DEFICITS IN A MOUSE MODEL OF RETT SYNDROME

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Rett syndrome (RTT) is a neurodevelopmental disorder that affects 10,000 to 15,000 live female births and is one of the leading genetic causes of severe mental retardation in females. Affected individuals typically display normal growth and development for a period of about 6-18 months during which they meet many normal developmental milestones. However, they then undergo a period of rapid regression, lose acquired skills, and often begin to exhibit autism-like features. Defects of the methyl-CpG-binding protein 2 (*MeCP2*) gene located on the X chromosome has been identified as the predominate cause of RTT. *MeCP2* is a nuclear protein that binds to methylated DNA which then represses gene expression through the recruitment of histone deacetylases. Catecholamine, dopamine and epinephrine, deficiencies have been reported in both RTT patients and mouse models. These deficits are implicated in respiratory dysfunction, anxiety issues, and motor impairments associated with RTT. Importantly, catecholaminergic cell specific ablation of *MeCP2* function was sufficient to produce phenotypes that are characteristic of RTT. Therefore, we hypothesized that restoration of

MeCP2 to catecholaminergic cells in an otherwise *MeCP2* deficient mice will improve various deficits. To test this, we produced rescue mice by crossing *MeCP2* deficient mice (*MeCP2* expression is disrupted by a floxed stop sequence) with mice expressing Cre recombinase driven by a tyrosine hydroxylase promoter. The animals were assayed for overall gross phenotypes as well as behaviour performances in the open field, light and dark placement preference, nest building, and accelerating rotarod tests. Restoration of *MeCP2* in catecholaminergic cells was sufficient to significantly improve life span and phenotypic severity. Anxiety-like behaviour, ambulatory activity, and motor deficits were also partially rescued. Taken together, the catecholaminergic system plays an important role in RTT pathophysiology and is a potential therapeutic target for RTT treatments.

SYSTEMIC INFLAMMATION ENHANCES GENERAL ANESTHETIC ENDPOINTS

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Introduction: General anesthetics produce multiple therapeutic endpoints including memory blockade, hypnosis, and immobility. Most surgical patients experience some degree of perioperative inflammation due to tissue injury or infection; however, whether inflammation influences the efficacy of general anesthetics is unknown. Here, we studied whether systemic inflammation modified the efficacy of the prototypical intravenous and inhalational general anesthetics, etomidate and isoflurane, for memory blockade, hypnosis, and immobility. Methods: REB approval was obtained. 3-4-month old, male mice (Sv129Ev x C57Bl/6) were treated with the endotoxin lipopolysaccharide (LPS) (125 µg/kg, i.p.) to induce systemic inflammation 3 hours prior to the administration of etomidate (6-30 mg/kg, i.p.), isoflurane (1.0-1.5%) or control vehicle. Memory blockade was studied using fear conditioning; hippocampus-dependent contextual fear memory and hippocampus-independent cued fear memory were assessed. Hypnosis was studied with the loss of righting reflex assay. Immobility was measured using the tail-pinch assay. Results: Contextual fear memory was reduced by etomidate in a dose-dependent manner, which was further reduced in LPS-treated mice compared with controls (two-way ANOVA, effect of etomidate, $F(2,42) = 6.39$, $P = 0.004$; effect of LPS, $F(1,42) = 26.79$, $P < 0.001$; etomidate × LPS, $F(2,42) = 0.36$, $P = 0.701$). Cued fear memory, in contrast, was not influenced by etomidate, but was still reduced in LPS-treated mice compared with controls (two-way ANOVA, effect of etomidate, $F(2,42) = 0.38$, $P = 0.683$; effect of LPS, $F(1,42) = 33.98$, $P < 0.001$; etomidate × LPS, $F(2,42) = 1.52$, $P = 0.230$). Latency to loss of righting reflex was reduced by etomidate or isoflurane in a dose-dependent manner, which was further diminished in LPS-treated mice compared with controls (two-way ANOVA, effect of etomidate, $F(2,43) = 21.23$, $P < 0.001$; effect of LPS, $F(1,43) = 19.20$, $P < 0.001$; effect of etomidate × LPS, $F(2,43) = 5.82$, $P = 0.006$; two-way ANOVA, effect of isoflurane, $F(2,42) = 50.41$, $P < 0.001$; effect of LPS, $F(1,42) = 22.26$, $P < 0.001$; isoflurane × LPS, $F(2, 42) = 2.31$, $P = 0.111$). Unresponsiveness to tail-pinch after etomidate (30 mg/kg) was increased in the LPS-treated group compared to control (Mann-Whitney U Test, 30 mg/kg, $P = 0.035$), whereas unresponsiveness to tail-pinch after isoflurane (1.39 ± 0.1%) was no different in LPS-treated and control groups (Student's t-test, $P = 1.000$). Conclusion: LPS further reduced contextual and cued fear memory induced by etomidate, further reduced latency to loss of righting reflex for etomidate and isoflurane, and increased unresponsiveness to tail-pinch for etomidate but not isoflurane. Collectively, the results show that inflammation causes alteration in anesthetic efficacy that is specific to each anesthetic and behavioural endpoint.

B.R.A.I.N.: Posters

B1. INCREASING CRTC1 FUNCTION IN THE DENTATE GYRUS DURING MEMORY FORMATION OR REACTIVATION INCREASES MEMORY STRENGTH WITHOUT COMPROMISING MEMORY QUALITY

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Previous research has established that the CREB (cAMP/Ca^{2+} responsive element binding protein) family of transcription factors is critical for memory in many species. CREB-regulated transcription co-activators (CRTCs; also known as Transducers of Regulated CREB activity, or TORCs) are latent cytoplasmic co-activators that shuttle to the nucleus in response to cAMP and calcium signals to stimulate CREB-mediated transcription in a CREB phosphorylation-independent manner. It has been suggested that, while there may be sufficient cellular levels of other CREB co-activators (CBP and p300) to support transcription, CRTC may be a limiting factor. The CRTC1 isoform is highly expressed in the rodent hippocampus and has been shown to stimulate CREB-dependent transcription and enhance synaptic plasticity. However, the role of CRTCs in memory is unknown. Here we examine whether acutely increasing CRTC1 levels in the dorsal hippocampus is sufficient to enhance the formation of context fear memory in mice. Recently, we found that CREB in the dorsal hippocampus is both necessary and sufficient for the formation of *spatial* memory. Here we show that increasing wild-type (WT) CRTC1 levels in the dorsal hippocampus is also sufficient to induce strong, precise *contextual fear* memory both at a recent and at a remote time point following weak training conditions that do not support contextual memory formation in WT mice. We also find that CRTC1 enhancement prior to context re-exposure enhances the reconsolidation of a remote context fear memory in WT mice. This is the first evidence demonstrating that CREB/CRTCs play a pivotal role in the dorsal hippocampus molecular machinery underlying the formation of contextual memory.

B2. NOVEL OPTOGENETICS APPROACH TO GENERATE *IN SITU* OXIDATIVE STRESS USING KILLERRED IN *C. ELEGANS*

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Optogenetics is a powerful approach to manipulate cell physiology by expressing genetically-encoded reagents in specific cells and activating these reagents by optical stimulation. Channelrhodopsin and Halorhodopsin are widely used to control membrane potential, but newer reagents are emerging to expand the applications of optogenetics to manipulate other cellular physiology. We established a novel method to use KillerRed (KR): a genetically-encoded red fluorescent protein that generates reactive oxygen species (ROS) upon light activation, to study oxidative stress in specific cells at specific timing.Oxidative stress has been implied in numerable diseases, including cancer, myocardial infarction, and neurodegenerative diseases, such as Parkinson's Disease (PD). Traditional approaches to induce oxidative stress involve whole-animal administration of chemical reagents, but chemicals diffuse into unexamined cells and often exert unintended side-effects. To directly address this problem, we expressed KR only in target cells, and by controlling excitation light of specific wavelength, we specified dose, timing, and duration to generate ROS. We employed the nematode *C. elegans* because of its transparent body, highly stereotypic anatomical positions of neurons, and powerful genetics; these advantages are ideal for using optogenetics in live animals. We imaged degenerating neurons over time in live animals, and upon KR-activation, 80.5±3% of KR-expressing neurons exhibited degeneration. Interestingly, the reduction in the neuronal function occurred before the distinct morphology changes, as assessed by behavioural assays.We also investigated dopaminergic (DA) neurons, which undergo degeneration in PD. We demonstrated light-induced oxidative stress in DA neurons, and observed ongoing neurodegeneration in live animals. By comparison of wild-type and mutant animals, we confirmed a protective role of LRRK2, a gene commonly mutated in familial PD, against cytosolic oxidative stress. Thus, we have established a novel method to address the role of oxidative stress in cell-specific manner, which can be applied to the study of other diseases.

B3. CHARACTERIZATION OF GLYCINE MEDIATED PRIMING IN GLUN1 SPLICE VARIANTS OF NMDARS

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NMDA (N-methyl-D-aspartate) receptors (NMDARs) are a principal subtype of excitatory ligand-gated ion channel with prominent roles in physiological and disease processes in the CNS. Previously, we discovered that stimulation of the glycine site independent of the glutamate site initiates signalling through the NMDAR complex, priming the receptors for clathrin-dependent endocytosis. Recently, we demonstrated that activation of the glycine site of NMDARs directly initiates transmembrane signaling events leading to recruitment of adaptin □2 (AP2) to recombinant NMDARs GluN1-1a with GluN2A or GluN2B expressed in HEK293 cells. The GluN1 gene contains various exons subject to alternative RNA splicing leading to the generation of eight splice variants. In the present study, we examined the effect of glycine stimulation on AP2 recruitment among GluN1 splice variants with either GluN2A or GluN2B subunits in HEK293 cell system. First, there was no difference in surface expression or basal AP2 association across the eight GluN1 splice variants. We next examined the effect of glycine on association of AP2 with NMDARs and found glycine stimulation increased the AP2 association with GluN1subunit, however, no change observed in cells express GluN1-1b variant. Furthermore, glycine stimulation did not cause a progressive reduction in NMDAR-mediated current in cells expressing GluN1-1b variants and no surface NMDAR endocytosis observed. Previously, we have shown recruitment of AP2 to NMDARs induced by glycine is required for subsequent receptor endocytosis upon binding of both agonists. Therefore, our results indicate the differences in glycine priming we observed among NMDAR splice variants also regulate the internalization of the receptors. It is known that the different GluN1 splice variant can interact with different binding partners and that these interactions can tightly regulate

intracellular internalization of the GluN1 subunits. Our observations also suggest that there may be unique roles for different GluN1 splice variants in glycine primed internalization of NMDARs.

B4. FATTY ACID SENSING THROUGH GPR120 IN HYPOTHALAMIC NEURONAL CELL LINES

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Fatty acids (FAs) modulate key signaling cascades regulating metabolism and energy homeostasis. For example, saturated FAs impair systemic glucose handling and alter orexogenic neuropeptide expression in the hypothalamus promoting pathogenic changes in insulin sensitivity and feeding behaviour. Conversely, unsaturated □-3 FAs can protect against such developments to prevent the subsequent onset of insulin resistance and type II diabetes mellitus (T2DM). In part, these effects are directly mediated through the G-protein coupled receptor 120 (GPR120), recently identified to bind □-3 FA to support insulin signalling in adipose tissue and fat handling in the liver [1]. However, the role for GPR120 in direct activation of hypothalamic neurons controlling feeding behaviour remains unknown. Here, we suggest a novel role for GPR120 in the hypothalamus, the key regulator of food intake and systemic energy levels. In clonal hypothalamic neurons immortalized from the embryonic rat (rHypoE-7), abundant GPR120 mRNA and protein expression was identified. GPR120 was functionally responsive to □-3 FA DHA and EPA in the hypothalamic cell models, and activation was concentration dependent (EC_{50} ~7-12 □M) and rapid (5 mins) as revealed by ERK and AKT activation using phospho-specific antibodies. As expected, no response was detected upon treatment with octanoic acid, a GPR120-independent FA, or in cell populations devoid of GPR120 protein. Prolonged activation of GPR120 (24 hours) reduced mRNA levels of the feeding neuropeptide agouti-related peptide (AgRP) as determined by quantitative real-time-PCR. Taken together, GPR120 activation by □-3 FA in hypothalamic neurons may compliment peripheral actions to support pathways pivotal in the prevention of obesity and the associated risk of T2DM. Future work will dissect the molecular mechanism of GPR120 dependent gene expression and delineate potential roles of GPR120 activation in hypothalamic insulin sensitivity. This study was supported by a BBDC Fellowship to LW and by CIHR, CRC, and CFI grants to DDB.

B5. MICROGLIA PODOSOMES: CHARACTERIZATION, REGULATION AND POTENTIAL ROLE IN MIGRATION

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Microglia are the resident immune cells of the central nervous system (CNS), which constantly survey the healthy CNS for 'stranger' and 'danger' signals. In response to CNS damage, microglia undergo a complex transformation process from a non-activated "resting" state to "activated". A crucial early response to most forms of CNS injury is migration of activated microglia to the damage site; however, little is known about mechanisms of microglia migration through the tightly packed extracellular matrix (ECM) in CNS tissue. We discovered that migrating microglia express many podosomes, often organized into a large ring in the lamella. Podosomes are one of two unique adhesion structures that have the capacity to degrade ECM; the second being the closely related invadopodia found in cancer cells. Podosomes have been identified in several cell types as small (0.5–1 □m) and dynamic structures (lifetime of 2–20 min) with an F-actin rich core surrounded by proteins, such as talin. Podosomes have been hypothesized to aid in localized substrate degradation and migration but have never been identified in any CNS cell type. Our extensive immunocytochemical characterization shows that podosomes in microglia express several molecules that are characteristic of podosomes in few other cell types previously examined, including F-actin, talin, Arp2/3 and Tks5. We found that podosome formation is regulated by Ca^{2+} entry through a specific store-operated Ca^{2+} channel, and we discovered several novel podosome components related to cellular Ca^{2+} signalling. We show that podosomes can degrade the ECM component, fibronectin, suggesting a role in migration. Together, this study provides the first evidence that microglia have podosomes, which might aid in ECM degradation and migration. In addition, we further characterize the structure, and potential signalling pathways in these unique subcellular structures.

B6. GROWTH-PROMOTING EFFECTS OF CARVACROL ON MOUSE HIPPOCAMPAL NEURONS

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Carvacrol is an essential oil found in herbs that has shown diverse cellular effects (e.g., antitumor, antimicrobial, and anti-inflammtory), but not much is known about its effect on neurons. It has recently been shown to inhibit mammalian TRPM7 channel. Transient Receptor Potential Melastatin 7 (TRPM7) channel is a ubiquitously expressed non-selective cation channel that has been shown to play a key role in anoxia- and ischemia-induced neurodegeneration. TRPM7 is suspected to be a secondary Ca^{2+} entry mechanism during cerebral ischemia. Transient knockdown of TRPM7 channels prevents neuronal death in the model of oxygen and glucose deprivation and global ischemia. Despite its detrimental role during cerebral ischemia, there is no selective pharmacological inhibitor that can specifically block TRPM7. Our objective is to examine the effect of carvacrol on hippocampal neurons in culture. Specifically, we investigated the chronic effect of carvacrol on embryonic neurons in culture over 6 days. We found that six hours after the application of carvacrol, DIV2 neurons treated with carvacrol (30-800 □M, 0.1% DMSO) had substantially longer total neurite length than control neurons. This effect of carvacrol is dose-dependent, with 200□M being the most effective dose. This neurite outgrowth promoting effect was also observed in DIV3 and DIV6 neurons treated with carvacrol. Furthermore, 6 and 24 hours following carvacrol-treatment, the longest neurite length of the treated neurons was significantly greater. Carvacrol also accelerated the maturation of the embryonic neurons in culture as that the neurons reached to the advanced developmental stages more quickly with carvacrol-treatment. Taken together, our findings suggest that low-dose carvacrol promotes neurite outgrowth in mouse hippocampal neurons. Additional studies using calcium imaging and patch-clamping will be carried out to elucidate whether observed effect

is due to carvacrol blocking TRPM7 channels.

B7. B-BAND ACTIVITY IN AND BETWEEN THE STN AND SNR OF PARKINSON'S DISEASE PATIENTS

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Parkinson's disease (PD) is accompanied by a significant amount of beta (☐)-band (11Hz-30Hz) neuronal and local field potential (LFP) oscillatory activity in the subthalamic nucleus (STN). Previous studies have shown significant coherence between neuronal firing and LFPs at ☐ frequencies at sites separated by ~1 mm and that the magnitude of ☐ oscillatory LFP activity and coherence are greatly reduced following levodopa administration. However, these data have been collected from large DBS contact electrodes or pairs of microelectrodes in proximity to each other and so it is not clear whether all regions of STN are synchronized. It is also not known whether the LFP and neuronal activity in the substantia nigra pars reticulata (SNr) shows ☐ activity and whether it is coherent with STN activity. Therefore, the aim of this study was to measure the spatial extent of ☐ coherent activity in the STN and SNr of PD patients OFF levodopa using dual microelectrode LFP recordings. A total of 109 pairs of recordings was collected from 9 patients undergoing DBS-STN implantation surgery. Trajectories passed dorsoventrally through STN and into SNr using two microelectrodes initially ~1 mm apart. Once the microelectrodes entered the dorsal STN, one of the two was held stationary, while the other one was advanced into SNr over a distance of ~ 4-6 mm. Pairs of recordings were obtained from STN/STN (n=55), STN/SNr (n= 47), and SNr/SNr (n= 7) at rest and during voluntary and isometric contractions of the forearm. We confirmed previous reports of a progressive attenuation in ☐ power as electrodes were driven from dorsal to ventral STN and into SNr. Furthermore, we found, significant ☐-LFP coherence across the dorsoventral extent of STN. Additionally, a significant coherence was found between ☐-LFPs located in dorsal STN and dorsal SNr. Although, isometric contraction decreased ☐-LFP peak amplitude, cell-cell ☐ coherence sometimes increased. These data suggest that the whole STN is entrained within the ☐ band in PD patients OFF meds. The finding of coherence between STN and SNr suggests that ☐ oscillations synchronize both the input and output nuclei of the basal ganglia.

B8. MOLECULAR INSIGHT INTO THE MECHANISMS UNDERLYING THE PLEIOTROPIC FUNCTIONS OF MUNC18-1 IN EXOCYTOSIS

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Synaptic transmission through the release of neurotransmitters (exocytosis) is critical for proper development and function of the brain. This process occurs through distinct stages: docking of vesicles to the plasma membrane, priming to a fusion competent state, and Ca^{2+} triggered membrane fusion. 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 suggest that Munc18-1 may act as a molecular chaperone of syntaxin-1, in priming of dense core vesicles (DCVs) and in docking of DCVs. Moreover, 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 play specific functions through distinctive binding modes with SNARE proteins; interaction between domain-1 and syntaxin-1 is critical for syntaxin-1 chaperoning and domain-3a mediates the priming stage of exocytosis through interaction with SNARE complex. 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. We have demonstrated that the domain-1 cleft of Munc18-1 is essential for high affinity syntaxin-1 binary interaction which is critical for syntaxin-1 chaperoning, DCV docking and consequently in secretion. Furthermore, we have discovered a novel mutant within domain-3a that exhibits an exciting phenotype. This "KE/5I mutant", which has a 5 residues insertion along with a K332E/K333E mutation can restore syntaxin-1 chaperoning function of Munc18-1 but fails to rescue secretion as assessed by immunoblotting, confocal microscopy, and biochemical secretion assay. Our data thus far suggests that domain-3a plays a critical role in the late stage of exocytosis beyond syntaxin-1 chaperoning. Our findings will provide significant mechanistic insight into the regulation of exocytosis by Munc18-1.

B9. THE TUBULIN-INTERACTING XENOPROTEIN CALTUBIN AS A NEUROREGENERATIVE TOOL

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The central nervous system (CNS) is frequently damaged as a result of traumatic injury or neurodegenerative disease. Unlike neurons of the peripheral nervous system (PNS), neurons of the CNS do not re-grow following injury. Thus far, the presence of CNS extracellular inhibitory factors has been considered a major factor in this discrepancy although it is not adequate to explain it completely. Growing evidence suggests that intrinsic outgrowth ability of central neurons can be enhanced to overcome inhibitory influences and permit modest outgrowth following injury. The novel EF-hand-containing protein caltubin is upregulated in CNS neurons of the regeneration-capable *Lymnaea stagnalis* (pond snail) following injury. It contributes to neurite outgrowth in central neurons of both *Lymnaea* and mice and is required for regeneration of adult CNS neurons in *Lymnaea (Nejatbakhsh et al., 2011 JNs)*. In mouse neurons, caltubin inhibits retraction following injury. Caltubin binds to tubulin and reduces its expression in both *Lymnaea* and mice, suggesting that it may affect outgrowth by modulating microtubule assembly. However, it is not yet known whether this interaction is required for caltubin's effects or which amino acids within caltubin encode its pro-regenerative function. In this study, we have established *in vitro* models to identify the functional peptide sequences of caltubin that underlie its effects in mouse central neurons and its

potential for regenerative effects *in vivo*. To evaluate function, we have created a number of eGFP-tagged reduced caltubin constructs and expressed them in this model to evaluate their role in the maturation and outgrowth of neonatal neurons. We also employed immunoprecipitation assays to identidy the interaction domains of caltubin and tubulin. These experiments provide preliminary evidence that elucidates the molecular mechanisms of caltubin as a potential pro-regenerative molecule in mammals.

B10. A RAT MODEL OF COMBINED STRIATAL STROKE AND ALZHEIMER'S DISEASE: SYNERGISTIC REDUCTION OF ADULT NEUROGENESIS IN THE HIPPOCAMPUS

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by a progressive decline in cognition and memory in the aging population. This progression is advanced by risk factors for vascular cognitive impairment, such as stroke. In this study, our team has generated animal models to investigate the underlying pathological interactions between stroke and AD. Four groups of young adult male Sprague-Dawley rats were used in the study: sham controls (n=6), AD model (n=8), stroke model (n=8) and the combined (AD & stroke) model (n=6). The cerebral focal ischemia (stroke) model was produced by a single injection of endothelin-1, a potent vasoconstrictor, into the right striatum. The AD model was created by bilateral injections of A□25-35 into the lateral ventricles. It has been previously shown that the combined AD & stroke model presented with a synergistic increase in inflammation and neurodegeneration in various brain areas. The present study further examines hippocampal adult neurogenesis in these animal models. Adult neurogenesis is crucial for certain types of learning and memory hence any changes in the rate of neural progenitor production or their survival could have consequence on cognitive impairment. Immunohistochemical markers of new neurons BrdU, Doublecortin, NeuN and Ki67 were used to quantify changes in neurogenesis. Stroke or AD treatment alone significantly impaired dendritic arborization (38% reduction in length) of young neurons 3 weeks after the surgery. The combined treatment further reduced neuronal survival by over 90%. This reduction in neuronal survival was not due to changes in cell proliferation at earlier time points. Our evidence suggests that combined stroke & AD can impact the survival of young neurons leading to more dramatic consequences in terms of possible cognitive decline. These effects on neurogenesis could contribute to the early onset and progression of Alzheimer's dementia.(This work is a collaborative effort carried out together by the Canadian Vascular and Cognitive Impairment Team.)

B11. ACCELERATED MATURATION OF EXCITATORY NEUROTRANSMISSION IN NMDAR KNOCK-DOWN MICE

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NMDARs play critical roles in regulating developmental plasticity in the mammalian brain, and are classically viewed as necessary for *de novo* synapses to recruit AMPARs into postsynaptic density (PSD) to strengthen synaptic efficacy and promote maturation. Previous studies have shown that postsynaptic NMDARs at a large axosomatic synapse, namely the calyx of Held-principal neuron synapse of the media nucleus of trapezoid body (MNTB) in the mouse auditory brainstem, are only present transiently. They are abundantly expressed from in early postnatal (P) days but rapidly down-regulated upon the onset of hearing at P11/12 to a very low or undetectable level after P16, raising the hypothesis that NMDARs are indispensable for inducing functional and morphological remodeling during the critical period of development. To test this, we examined the properties of the calyx of Held-MNTB synapses *in vitro* in slices from juvenile NR1 know-down (NR1-KD) mice and wild-type (WT) littermates, and auditory brainstem responses (ABRs) *in vivo*. By whole-cell voltage-clamp recordings from MNTB neurons, we revealed a significant reduction in the current amplitude of NMDAR-EPSCs by up to 70%, but surprisingly no difference in that of AMPAR-EPSCs between NR1-KD and WT mice before P11/12. Interestingly, AMPAR-EPSCs are significantly increased by 15% - 30% ($P<0.05$) after the onset of hearing in NR1-KD mice. High-frequency stimulation (100 Hz, 100 ms) led to more robust short-term depression (STD) in NR1-KD synapses than WT at P9/10 ($P<0.05$) but this difference in STD diminished and eventually reversed after P15. In parallel, recovery from STD accelerated with much faster time course in NR1-KD synapses than WT synapses, indicating a slowed depletion and accelerated replenishment of the readily-releasable pool of synaptic vesicles in NR1-KD synapses. These changes take place in the absence of gross morphological differences between two groups, as evidenced by fluorescence labeling of presynaptic calyces. These functional profiles in NR1-KD mice are very much reminiscent of those in more mature WT mice, suggesting a novel role for NMDARs to function as an activity-dependent control (or brake) for setting the pace of synaptic consolidation and maturation in the mammalian brain.

B12. NCS-1 PROTEIN UPREGULATION IS NECESSARY FOR CHRONIC HYPOXIA-INDUCED RESPIRATORY ADAPTATION

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NCS-1 belongs to the neuronal calcium sensor (NCS) family of calcium (Ca) binding proteins. Recent studies have shown that upregulation of NCS-1 in stressful conditions (e.g., H_2O_2, axotomy, high Ca) confer protection to the exposed neuronal cells. In plants, NCS-1 is necessary for low pH tolerance, and in human brains, levels of NCS-1 are correlated with the acidity of brain tissue. Another closely related member of the NCS family, calsenilin (DREAM/KChIP), has been shown to promote cell death in cultured neurons during hypoxia. These evidences lead to the hypothesis that NCS-1 may play a significant role during hypoxia. To test this hypothesis, we used the hypoxia-tolerant freshwater pond snail, Lymnaea stagnalis to evaluate the correlation between NCS-1 and hypoxia induced behaviour changes. Our results show that chronic hypoxia treatment facilitates aerial respiratory behaviour in snails. Furthermore, ganglionic NCS-1 protein level is upregulated following chronic hypoxia treatment. At the cellular level, immunocytochemistry showed that NCS-1 was up-regulated in a respiratory pacemaker CPG neuron, right pedal dorsal 1 (RPeD1) under hypoxia condition. Behaviourally, hypoxia-induced facilitation of aerial respiratory activity was

attenuated with the application of NCS-1 siRNA before chronic hypoxia treatment. Furthermore, NCS-1 siRNA also inhibits the hypoxia-induced changes in respiratory pacemaker neuron activity. These preliminary results suggest that NCS-1 upregulation during chronic hypoxia is necessary for adaptation to chronic low oxygen levels.

B13. N-TERMINAL PEPTIDE OF SYNTAXIN-1 SECURES ITS INTERACTION WITH MUNC18-1 FOR TRAFFICKING.

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Neurotransmitter release is a key process in communication between neurons. Upon calcium influx, neurotransmitters stored in vesicles are released from neurons. The fusion of vesicles with the plasma membrane is believed to be executed by the SNARE (Soluble N-ethylmaleimide sensitive factor Attachment protein REceptors) complex comprising syntaxin-1, SNAP-25, and VAMP2. Numerous studies have provided evidence suggesting that syntaxin-1 is a critical protein for neuronal exocytosis. Nevertheless, the role of syntaxin-1 in mammalian systems remains unclear due to the presence of its two isoforms, both of which need to be removed in order to elucidate the protein function. Moreover, the physiological role of the interaction between syntaxin-1 and Munc18-1, an indispensible protein in neurotransmitter release, is in question. Several studies have proposed that N-terminal peptide of syntaxin-1 is essential for the acceleration of SNARE mediated fusion by Munc18-1. However, we hypothesize that the N-terminal peptide is critical for securing the interaction of syntaxin-1 and Munc18-1. In order to test this hypothesis, PC12 cells depleted of both syntaxin-1A and 1B isoforms were generated and these cells exhibited a severely defected secretory phenotype. Subsequently, the syntaxin-1 knockdown cells were engineered to express mutant forms of syntaxin-1A that carry point mutations introduced into the N-terminal peptide. After secretion analysis, we found that some mutant expressing cells were not able to rescue defected secretion and interestingly, these cells displayed severe accumulation of the proteins in intracellular compartments. Considering that the interaction of syntaxin-1 and Munc18-1 has been shown to play a role in trafficking of syntaxin-1 to the plasma membrane, the lack of secretion rescue by some mutants can be explained by their mislocalization caused by impaired interaction with Munc18-1. Thus, our data suggest that the N-terminal peptide of syntaxin-1 secures the binary interaction between syntaxin-1 and Munc18-1 for the proper localization of syntaxin-1.

B14. SIRT3 OVEREXPRESSION PROTECTS DIFFERENTIATED PC12 CELLS FROM GLUCOSE DEPRIVATION OR OXYGEN-GLUCOSE DEPRIVATION.

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SIRT3 is a mammalian sirtuin targeted to mitochondria. In non-neuronal cells SIRT3 over-expression increases cellular respiration efficiency, and decreases levels of reactive oxygen species. SIRT3 is present in the brain but there is little data on SIRT3 in neurons. We hypothesized that over-expression of SIRT3 would be neuroprotective and diminish cell vulnerability to oxidative stress. Differentiated PC12 cells were transfected with pTracer-CMV2-SIRT3 plasmid; transfection efficiency was 43.4 ± 2.7% after 24h. MTT reduction assays showed that cell viability was decreased for the first 4 days post-transfection but subsequently recovered. Confocal imaging using Rhodamine123 revealed that mitochondrial membrane potential was decreased in SIRT3 overexpressing cells but mitochondrial morphology was normal. PC12 cells were challenged with glucose deprivation (GD) or oxygen-glucose deprivation (OGD). GD was induced by incubating cells for 4 hours in glucose/glutamine deficient medium (-/- medium) plus 10mM 2deoxy-D-glucose (2DG) and 10□M CCCP. To induce OGD cells were incubated with (-/-) medium plus 2DG, and placed in an anoxic environment for 5 hours. Cell death was quantified using confocal microscopy and PI as follows: % of dead transfected cells = (# of dead (PI+) transfected cells/ # of transfected cells) x 100%. SIRT3 overexpression was neuroprotective for GD; after a GD challenge followed by 15 hrs reperfusion (RP), cell death was 39.7 ± 9.3 % in SIRT-3 overexpressing cells versus 72.5 ± 9 % in controls transfected with plckGFP. SIRT3 overexpression also reduced cell death after OGD; 20 hrs post OGD/RP cell death in SIRT3 transfected cells was significantly less (10.4±5.1%) than in controls transfected with plckGFP (32.0 ±11.7%). These data are the first evidence that SIRT3 over-expression is protective in a neuronal model of oxidative stress.

B15. THE ROLE OF REPULSIVE GUIDANCE MOLECULE B (RGMB) IN THE DEVELOPING CHICK VISUAL SYSTEM.

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The Repulsive Guidance Molecules (RGMs) are a family of membrane proteins of which three members, RGMa, RGMb, and RGMc have been identified. In the developing chick visual system, RGMb is expressed in retinal ganglion cells and in the optic tectum. Furthermore, RGMb interacts with proteins that are involved in retino-tectal patterning and axonal development, including Bone Morphogenetic Proteins (BMPs) and Neogenin. It is therefore hypothesized that RGMb has an important effect on retino-tectal patterning and axonal development in the developing chick visual system. To determine the role of RGMb, various DNA constructs were developed to i) over-express RGMb in the developing chick eye and ii) knock down RGMb expression in this system. In order to knock down RGMb, microRNA (miRNA) viruses against RGMb were injected into the neural pore *in ovo* at embryonic day 2 (E2). A DiI crystal is implanted at E15. This crystal traces the path of retinal axons through the optic nerve and the termination in the optic tectum. Thus far, when RGMb is knocked down, aberrant nerve projections are observed. Additionally, it is hypothesized that RGMb is involved in retinal ganglion cell axon projection through its interaction with Neogenin. Through binding assay analysis, RGMb is shown to specifically bind to the fibronectin III – 3,4 (FNIII-3,4) domain of Neogenin. This novel finding has helped determine the mechanism through which RGMb acts to exert its repulsive guidance effects. Determining the function of RGMb in the developing chick visual system will enhance our understanding of

the mechanisms of axonal development from the retina to the optic tectum along with the novel roles that RGMb may have. Such data will also provide a useful model for axon development in the central nervous system as a whole.

B16. THE EFFECTS OF DISRUPTING DAT-D2 DIRECT PROTEIN-PROTEIN INTERACTION IN DOPAMINERGIC NEUROTRANSMISSION: A POTENTIAL THERAPEUTIC TARGET FOR PD.

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Parkinson's disease (PD) is a neurodegenerative disorder with motor abnormalities such as bradykinesia, muscular rigidity, postural instability and tremor at rest. It is commonly accepted that the etiology of PD is primarily due to the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta. Such neuronal loss leads to reduced dopaminergic transmission within the nigrostriatal pathway, severely affecting the motor circuitry. Given the abnormality underlying PD, any measures that can increase synaptic dopamine, especially in the nigrostriatal pathway, would in principle be able to alleviate the motor symptoms of PD. We discovered a direct protein-protein interaction between DAT and the short variant of D2 receptor (D2S). At the cellular level, this interaction allowed D2S to elevate the expression DAT on the plasma membrane, thereby enhancing the DAT-mediated DA uptake. Subsequently, we showed that a truncated DAT peptide (M_1-V_{15}) was able to disrupt this D2S-DAT interaction, thereby salvaging the synaptic level of dopamine. Furthermore, we hypothesized that the disruption of the DAT-D2S interaction would suffice to improve motor performance in DA-depleted or PD animal models. In open-field locomotor test, the DAT peptide showed to improve locomotion in rats with □-methyl-p-tyrosine-induced DA depletion ($p < 0.05$). Meanwhile, in the cylinder test, the DAT peptide alleviated forelimb asymmetry in 6-hydroxydopamine (6-OHDA) lesioned rats ($p < 0.05$).Based on the findings in this study, we concluded that the disruption of DAT-D2S interaction can serve as a potential target site in regards to developing treatments for PD.

B17. THE INTERACTION BETWEEN DOPAMINE D5 RECEPTOR AND PLASMA MEMBRANE CALCIUM ATPase

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The concentration of calcium within cells is crucial to many physiological processes. The plasma membrane Na^+ / Ca^{2+} exchanger and the Plasma Membrane Calcium ATPase (PMCA) are the two main mechanisms responsible for removing calcium from cells. Recently, we have found a potential role for the dopamine D5 receptor, a D1-like dopamine receptor, in PMCA regulation. Dopamine is a catecholamine neurotransmitter that plays a major role in regulating motor function, emotion and reward. We found that PMCA and D5R can form a complex in rat brain and kidney, through which D5R can regulate PMCA function. More interestingly, we observed that the D5R-PMCA interaction was significantly enhanced in the spontaneous hypertensive rat (SHR) kidney. The aim of this project is to characterize the protein-protein interaction between PMCA and D5R, to investigate whether the D5R-PMCA interaction is responsible for D5R-mediated modulation of PMCA's ability to extrude calcium; and to investigate the physiological relevance of the D5R-PMCA interaction. An in-vitro binding assay and subsequent silver staining of SDS-PAGE to probe for direct protein-protein interactions revealed a 20 amino acid sequence within the C-terminal of D5R that directly interacts with PMCA. Furthermore, using immunofluorescence assays, we have shown that these two proteins co-localize when both are expressed in HEK293 cells. In HEK293 cells expressing D5R and PMCA, we have shown that D5R activation with a dopamine agonist results in a statistically significant increase in PMCA's ability to remove calcium from cells. Furthermore, expression of a mini-gene coding the 20 amino acids in the D5R C-Terminal necessary for this interaction was able to block the interaction between D5R and PMCA. Ultimately, we will generate a small peptide that is capable of disrupting the interaction between D5R and PMCA. Since the interaction between D5R and PMCA is enhanced in the spontaneous hypertensive rat kidney, a peptide that blocks the interaction between D5R and PMCA will allow us to examine the physiological and/or pathological role of the interaction between D5R and PMCA.

B18. ABERRANT REGULATION OF PREFRONTAL 5-HT_{1A} RECEPTORS BY TYROSINE PHOSPHORYLATION IN A MOUSE MODEL OF DEPRESSION

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Both in developmental and in adulthood, the serotonin transporter (5-HTT) is important for establishing normal emotional behaviors. Specifically, constitutively-decreased transcription of the 5-HTT gene has been shown to increase anxiety and depressive behaviors in humans as well as rodents. Since serotonin signaling in the prefrontal cortex plays a central role in emotional responses, we examined whether mice with reduced 5-HTT transcription (5-$HTT^{+/-}$ mice) display altered serotonin receptor function in the prefrontal cortex. Performing whole-cell electrophysiological recordings in acute brain slices, we found that adult 5-$HTT^{+/-}$ mice displayed an increase in the magnitude of inhibitory 5-HT_{1A} currents in the major output neurons of the prefrontal cortex. This increase was relative to wildtype sibling controls and was similar to that observed in the full knockout 5-$HTT^{-/-}$ siblings. Since developmental alterations in the 5-HTT can result in persisting changes to brain and behaviour, we examined whether pharmacological inhibition of the 5-HTT during development was sufficient to elicit this increase in the prefrontal 5-HT_{1A} receptor currents. We found that wildtype mice treated during the early postnatal period with fluoxetine (PN-Flx) displayed a similar increase in prefrontal 5-HT_{1A} currents to the 5-$HTT^{+/-}$ mice when examined in adulthood. Next, we examined the cellular pathway responsible for regulating the 5-HT_{1A} receptor current. In adult wildtype mice, we found that the 5-HT_{1A} receptors are normally under tonic inhibition by Src family tyrosine kinase phosphorylation, with little opposing activity of the tyrosine phosphatase. In 5-$HTT^{+/-}$ and PN-Flx mice, however, this phosphorylation-mediated inhibition appears to be substantially reduced, leading to the increase in 5-HT_{1A} receptor currents. Together, these findings demonstrate that prefrontal 5-HT_{1A} receptors are regulated by tyrosine phosphorylation and suggest that deregulation of this pathway

may contribute to the prefrontal cortical dysfunction observed in mood disorders.This research was supported by grants from NSERC (EL) and the Scottish Rite Charitable Foundation (EL) and a CHIR Banting and Best Doctoral Award (NG).

B19. BASKET CELL CONTRIBUTIONS TO THE GENERATION OF THETA RHYTHMS IN MODEL HIPPOCAMPAL CA1 NETWORKS

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Theta rhythms are one of the most prominent and well-studied clocking mechanisms detected in the mammalian brain. These 3-12 Hz oscillations are thought to play a lead role in spatial navigation and episodic memory, and are recorded from the hippocampus during R.E.M. sleep and exploratory behavior [1]. Although theta rhythms have been heavily studied, the mechanism(s) responsible for their generation remains unknown. Recent research shows that in an intact hippocampus *in vitro,* the CA1 hippocampal region possesses the necessary circuitry to generate intrinsic theta rhythms [2]. To determine the mechanism(s) underlying the generation of these CA1 theta rhythms, we combined experimental recordings, data analysis, and mathematical modeling. Our mathematical network model is composed of four types of cells: pyramidal cells, parvalbumin-positive basket cells (PV+BCs), cholecystokinin-positive basket cells (CCK+BCs), and oriens – lacunosum-moleculare (O-LM) interneurons. Each cell type is represented by a single-compartment model, and the network architecture is based on experimental data of known connectivities. Intracellular data recorded from the CA1 region of the intact hippocampus *in vitro* was used in combination with mathematical extraction techniques [3,4] to determine the balance of synaptic excitation and inhibition in PV+BCs and O-LM interneurons during the theta rhythm. Our network model produces robust theta rhythms and cellular phase relationships in accordance with the experimental data. Research has focused on the role of PV+BCs in faster gamma rhythms (20-100 Hz), yet our model surprisingly finds that the PV+BCs play a critical role in the production of these slower theta rhythms. In addition, mean synaptic conductance values extracted from intracellular recordings indicate that during the quiescent state of the CA1 theta rhythm, PV+BCs receive more excitatory and inhibitory synaptic input than putative O-LM interneurons. Optogenetics will be used to test predictions about the role of individual interneuron types in the generation of CA1 hippocampal theta rhythms. This work was supported by the Canadian Institutes of Health Research and the *Natural Sciences and Engineering Research Council* of Canada

B20. HDAC6 INHIBITION AS AN ANTI-EPILEPTIC MECHANISM IN A MOUSE MODEL OF RETT SYNDROME

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Seizures have been ranked as the co-morbidity most significantly affecting the quality of life of Rett syndrome patients. These seizures tend to be poorly controlled by classic anti-convulsive drugs, highlighting the need to identify mechanisms of seizure genesis and develop new methods of seizure prevention. The histone deacetylase-6 complex (HDAC6) represents an intriguing candidate system that could play a role in Rett syndrome pathophysiology. We have found HDAC6 to be abnormally higher in MeCP2-defecient mice and increased HDAC6 has been directly shown to impair intracellular transport, specifically in neurons. It has also been noted that inhibiting HDAC6 has been shown to restore normal transporting capabilities. Given this, we hypothesized that HDAC6 inhibition would improve the behavioural and neurophysiological deficits of MeCP2-deficient mice through restoration of normal intracellular transporting capabilities. To test this hypothesis, female MeCP2-/+ mice were injected daily with a novel HDAC6 inhibitor, and their cortical EEG and general behavioural performances were compared with pre-treatment levels. HDAC6 inhibition significantly reduced the rate of epileptiform discharges in MeCP2-/+ mice, and markedly improved their performance on the Accelerating Rotarod test. Additionally, acetylated-□-tubulin, an indicator of intracellular transport, was found to be significantly increased in Tubastatin treated MeCP2-deficient mice. Taken together, these results implicate HDAC6 complexes in the etiology of Rett syndrome, and provide direct evidence that diminishing HDAC6 activity improves the epilepsy phenotype of adult MeCP2-deficient mice. Collectively, this study supports the further investigation of HDAC6 inhibitors as potential treatment strategies for Rett syndrome.

B21. USING MODEL DATABASES TO DETERMINE DENDRITIC DISTRIBUTIONS OF I_H CHANNELS IN ORIENS-LACUNOSUM/MOLECULARE HIPPOCAMPAL INTERNEURONS

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The hippocampus is an important region of the brain that is critically involved in learning and memory, spatial navigation, and exploratory movements. Inhibitory interneurons are known to be crucial in the generation of population rhythms that are expressed during these behavioural states. One interneuron type is the stratum oriens-lacunosum/moleculare (O-LM) cell, which regulates pyramidal cell activity via its distal dendrites. To understand their contribution to hippocampal output, it is necessary to investigate their activities both intrinsically and in network contexts. To do this, the use of biologically grounded, multi-compartment computational models is needed since they provide the ability to examine the simultaneous interaction of multiple conductances. However, due to the variability of experimental data, developing a set of models that collectively capture O-LM cell behaviour is required. The goal of this research is to develop a model database to determine possible distributions of hyperpolarization-activated cation currents (I_h) in

O-LM cell dendrites, which is currently unknown. I_h currents are known to have pacemaking roles, and their presence on O-LM cell dendrites could directly influence incoming synaptic input. Physiologically plausible ranges for conductance densities in the models have been specified, and simulations have been executed on the SciNet cluster. Electrophysiological recordings of O-LM cells in mice have been obtained and analyzed using the PANDORA software toolbox. This data has been applied to generate a database of appropriate O-LM models, a subset of which has been quantitatively compared to a subset of the electrophysiological data using PANDORA. Our analysis currently indicates that models with I_h both in the soma and dendrite, rather than just in the soma, more closely conform to experiment. Our work therefore suggests that I_h could directly modulate incoming synaptic signals by their dendritic location, thus affecting the contribution of O-LM cells to hippocampal network rhythms.

B22. THE ROLE OF PUM2 IN AXONAL OUTGROWTH

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Pumilio 2 (Pum2) is a homologue of drosophila Pumilio: a founding member of the *Puf* family of mRNA binding proteins and translational regulators. These proteins have been shown to be important for proper embryonic development and are involved in the development and function of various systems, such as the reproductive and nervous systems. Furthermore, Pum2 has been shown to regulate the level and the activity of the eukaryotic initiation factor 4E (eIF4E). The purpose of this study is to characterize the role of Pum2 in axonal outgrowth. To examine the role of Pum2 in axonal outgrowth, dorsal root ganglia (DRG) neurons were electroporated with cDNA constructs that overexpress a wildtype Pum2 (Pum2EYFP), a dominant negative mutant (Pum2G947D), another mutant (Pum2W349G), or two silencing constructs (Pum2miRNA1 and Pum2miRNA2221). When Pum2 is overexpressed in DRG neurons, the length of neurites is approximately 84.7±2.7% ($p < 0.0001$) and 64.2±8.2% ($p = 0.001$) shorter when compared to control GFP and Pum2G947D transfected neurons, respectively. When Pum2W349G mutant is overexpressed, the length of the neurites is approximately 85±1.7% shorter than the control ($p<0.001$). Pum2 silencing using Pum2miRNA1 and Pum2miRNA2221 constructs shows a 60±10% ($p=0.016$) and a 60±8% ($p<0.01$) reduction in neurite length, respectively. Co-overexpression of eIF4E and Pum2 is being examined for a possible rescue of the short neurite phenotype. These data indicate a significant involvement of Pum2 in axonal outgrowth. More experiments are underway to investigate other functional aspects of Pum2 in this process.

B23. CHARACTERIZATION OF SEVERE HYPOGLYCEMIC SEIZURES IN JUVENILE DIABETIC RATS

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Human Type I diabetics receiving insulin treatment are at significant risk for repeated severe hypoglycemic episodes, often with seizures, and more rarely, the catastrophic "dead in bed" syndrome. To our knowledge, this is the first study that systematically investigates the impact of a severe hypoglycemic episode in juvenile diabetic animals; assessing pathophysiology and potential treatment strategies. Postnatal 21 day old male Sprague Dawley rats were treated with streptozotocin (STZ), a ßcell-specific toxin, at the optimized dose of 80mg/kg to produce the type 1 diabetes phenotype. Diabetic animals demonstrated elevated blood glucose levels (above 11.1 mmol) within 7 days and delayed weight gain, both factors which persisted up to 2 months of age. At 28 days, diabetic animals (n=69) were fasted overnight, injected with insulin, 15 u/kg i.p., and monitored for seizure-like activity. Repeated behavioral seizures were observed in 43 hypoglycemic animals and the rate of mortality was 37%. There was no mortality in hypoglycemic animals that did not exhibit seizure behaviour. The average latency to seizures in diabetics was 2.4±0.16 hours with blood glucose levels at seizure onset reaching an average of 1.78±0.16mmol. Glucose administration alone even when repeated was not always successful in preventing subsequent seizures. Treatment at seizure onset i.p. with 500ug of diazepam, 5mg of phenytoin and 0.5ml of 25% glucose reduced the incidence of subsequent seizures in 71% (n=21) of rats compared with 45% (n=22) treated with glucose alone ($p<0.05$). Intracranial electroencephalograms (EEG) were performed in awake, free-moving hypoglycemic rats (n=9) in the CA1 as well as cortex and MRF regions. While all animals showed changes in EEG, 2 rats showed EEG suppression. Motor seizures were observed in 5 of 9 rats; however there was no ictal activity suggesting the seizures might originate from subcortical regions.

B24. PROBING THE MECHANISMS UNDERLYING GABAPENTIN'S ANALGESIC AND ANXIOLYTIC PROPERTIES.

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Gabapentin is widely prescribed for its analgesic, anxiolytic, hypnotic and sedative properties. The mechanism(s) underlying these various therapeutic effects remain poorly understood. Gabapentin modulates the □2□-1 subunit of voltage-dependent calcium channels; however, this interaction fails to fully account for the behavioral effects of gabapentin. We previously showed that gabapentin increases a tonic inhibitory conductance in mammalian neurons that is generated, in part, by d subunit-containing $GABA_A$ receptors ($dGABA_ARs$). Also, $dGABA_ARs$ are effective regulators of nociceptive processes and hence, are novel targets for analgesics. The aims of the current study were to determine, whether gabapentin increases the concentration of $dGABA_ARs$ agonists including GABA and other ligands and, whether the anxiolytic and analgesic properties of gabapentin are reduced in genetically-modified mice that lack $dGABA_ARs$ (*Gabrd-/-*). The studies were approved by the local animal care committee. The concentrations of agonists in tissue obtained from gabapentin-treated mice and cultured neurons were measured using mass spectroscopy. Anxiety levels and anti-nociceptive effects in gabapentin (30mg/kg)-treated or vehicle-treated wild type (*Gabrd+/+*) mice and *Gabrd-/-* mice were probed using the elevated plus maze and formalin assay,

respectively. Gabapentin did not increase the level of GABA or other ligands. Gabapentin caused anxiolysis in *Gabrd+/+* mice but not *Gabrd-/-* mice as evidenced by an increase in the percentage time spent in the open arms (*Gabrd+/+*: 11.3 ± 3.4%, n = 10; *Gabrd-/-*: 3.2 ± 1.1% n = 11, P < 0.01). The results of studies of the nociceptive effects of gabapentin are pending. Collectively, the results show that gabapentin does not increase the concentration of agonists that activate $dGABA_ARs$ suggesting that function or expression levels of these receptors are increased. $dGABA_ARs$ are identified as novel targets that contribute to the anxiolytic properties of gabapentin. These results are of interest given the alterations of $dGABA_ARs$ under a variety of pathophysiological states.

B25. REPISRATORY FUNCTION IMPROVES WITH TRUNK MUSCLE STIMULATION AMONG INDIVIDUALS WITH SPINAL CORD INJURY

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Pathological breathing following spinal cord injury (SCI) is dependent on the level and completeness of injury. Multi-system motor dysfunction, including incoordination of respiratory muscles and postural instability, inhibits proper diaphragmatic function in upright sitting resulting in poor expiratory lung capacity and reduced tidal volume. Functional electrical stimulation (FES) improves sitting posture, including pelvic orientation and spinal alignment, and lung capacity during a forced expiratory manoeuvre. However, quiet breathing respiratory function (RF_Q) remains to be investigated among individuals with SCI during application of FES. Our objective was two-fold: 1) to describe the effects of posture on RF_Q during quiet sitting in able-bodied individuals; and 2) to test the feasibility of using FES to improve RF_Q in individuals with SCI. FES was tested in two arrangements: bilateral stimulation of rectus abdominis and lumbar erector spinae for anterior-posterior (AP-FES) and obliquus externus and latisimus doris for medial-lateral (ML-FES). First, ten able-bodied subjects (ABs) were asked to maintain quiet sitting balance in upright (Sit_{UP}) and slouch (Sit_{SL}) postures. Next, three subjects with SCI were asked to maintain quiet sitting balance while sitting: unsupported (**Sit_{UN}**); assisted by AP-FES; and assisted by ML-FES. RF_Q measures – tidal volume, V_T (l); respiratory rate, RR (min^{-1}); and minute ventilation, V_E (l/min) – were obtained for both groups. Among ABs, Sit_{UP} was associated with greater respiratory volumes, but not greater breathing frequency. During Sit_{UP}, V_T increased by 14% compared to Sit_{SL} ($p<0.01$); RR was not changed between Sit_{UP} and Sit_{SL} ($p=0.18$). Overall, V_E was 6% greater during Sit_{UP} compared to Sit_{SL} ($p<0.05$). In SCI case studies, there was an overall trend toward greater V_E with FES, compared to Sit_{UN}: in 2/3 cases, V_E increased by more than 10% due to bidirectional changes in both V_T and RR. The overall increase in V_E in ABs during upright sitting may be due to optimal respiratory mechanics: as posture may optimize mechanical function of the diaphragm to achieve peak quiet breathing **V_T**, the RR remains stable between upright and slouch postures. Among SCI cases, there was a trend toward improved RF_Q indicated by increased **V_E** with FES. These results will facilitate an understanding of the link between postural control and RF_Q in individuals with SCI**.**

B26. PREFRONTAL MUSCARINIC CURRENTS ARE POTENTIATED BY Ca^{2+} RELEASE FROM INTRACELLULAR STORES: INVESTIGATING A ROLE FOR ELECTROGENIC Ca^{2+} EXTRUSION

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Acetylcholine (ACh) plays a key role in modulating prefrontal cortex function. In layer V pyramidal cells, it activates Ga_q-protein coupled M1 muscarinic receptors, resulting in a slow excitatory inward current as well as in the release of calcium (Ca^{2+}) ions from internal stores. Preliminary findings suggest that this Ca^{2+} release may increase the magnitude of the inward current. We hypothesized that the larger current results from the activation of an electrogenic sodium-calcium exchanger to extrude the Ca^{2+} released from intracellular stores. To investigate this phenomenon, we examined both effects with simultaneous whole cell voltage-clamp electrophysiological recordings and multiphoton calcium imaging of layer V prefrontal neurons in acute brain slices from adult rats. Bath applications of ACh (15 s) reliably elicited inward currents in the recorded cells (Vh = -75 mV) but only elicited somatic calcium responses in ~50% of neurons. A brief depolarizing step applied at baseline could effectively "prime" non-responders such that subsequently-applied ACh elicited a calcium response. In within-cell and cross-sectional experiments, excitatory muscarinic currents were significantly larger when accompanied by calcium increases. Depleting calcium stores with thapsigargin significantly reduced both the ACh-elicited calcium responses and inward currents. Next, we performed ion substitution experiments to probe for involvement of an electrogenic exchanger and found that the ACh-elicited inward currents were almost completely abolished by 80% extracellular sodium (Na^+) substitution with NMDG or choline, a manipulation that did not alter the amplitude nor kinetics of the calcium response. By contrast, elimination of both intracellular and extracellular K^+ significantly enhanced the amplitude and duration of calcium responses and also enhanced the inward currents. These data suggest that calcium responses are shaped by a K^+-dependent clearance mechanism and that the calcium-potentiated M1 muscarinic currents are Na^+-dependent. Further experiments will test the involvement of the sodium-calcium (NCX) and sodium-calcium-potassium (NCKX) exchanger proteins in mediating the potentiation of muscarinic currents resulting from release of calcium from intracellular stores.

B27. CALCIUM, CALPAIN, AND CALCINEURIN IN LOW-FREQUENCY DEPRESSION

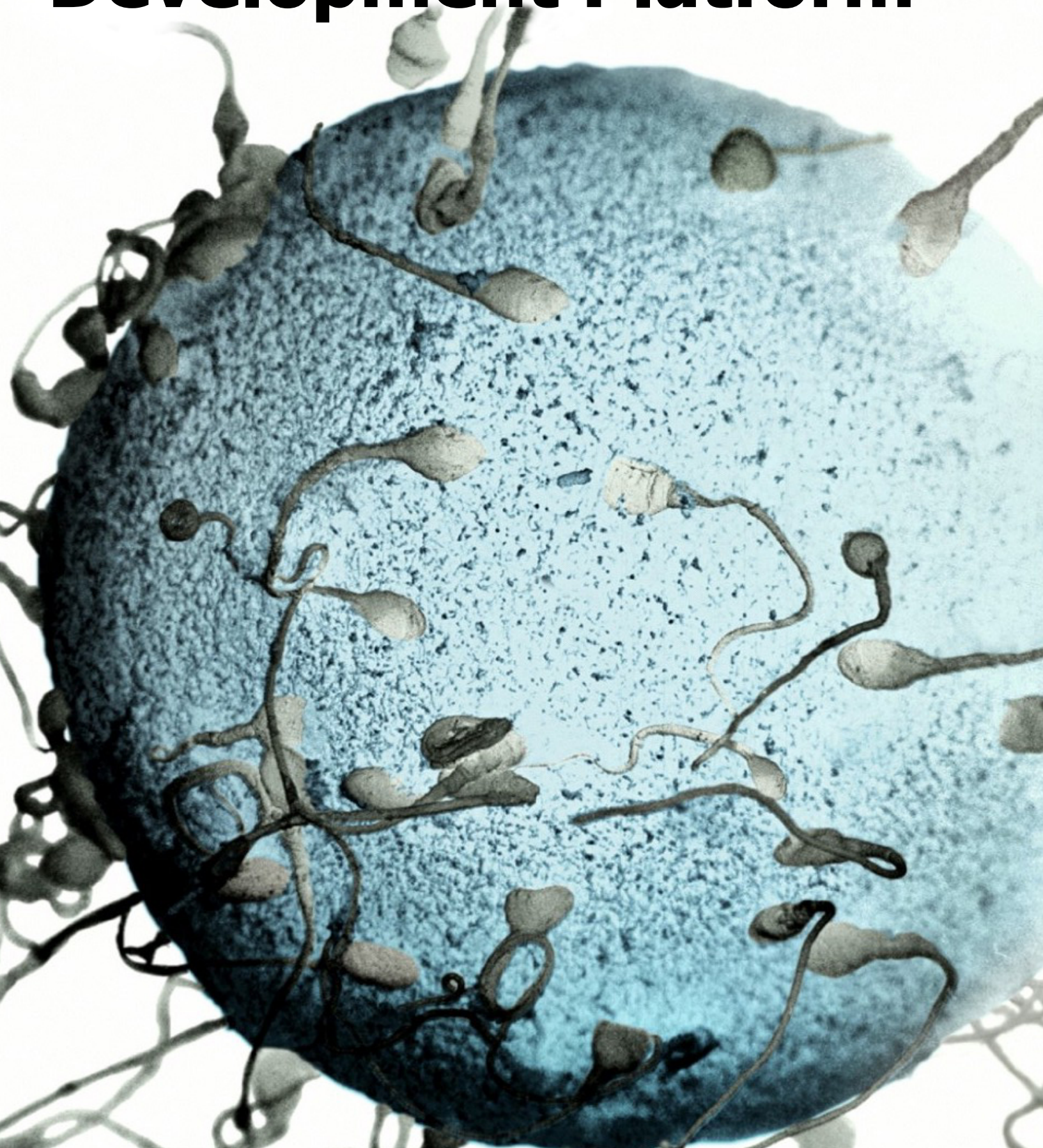
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Transmitter release at crayfish leg extensor neuromuscular junction (NMJ) phasic synapses declines by over 50% in 60 min at low frequency stimulation with 0.2 Hz in both dissected preparations and in free living animals. This low-frequency depression (LFD) is regulated by protein phosphorylation by PKA and PKC and dephosphorylation by protein phosphatases 1, 2A and Ca2+ activated phosphatase 2B (calcineurin) (Silverman-

Gavrila et al., 2005). Presynaptic calcineurin activity is necessary for LFD (Silverman-Gavrila and Charlton, J. Neurochemistry, 2009). Since the fast Ca2+ chelator BAPTA-AM inhibits LFD but the slow chelator EGTA-AM does not, depression is not caused by widespread residual free calcium in the presynaptic terminals, but rather the Ca2+ sensor for LFD may be close to a Ca2+ source such as Ca2+ channels where a large brief Ca2+ signal is available at active zones. Alternatively, calcineurin could be constitutively activated in response to limited proteolysis of the autoinhibitory domain of calcineurin by Ca2+-dependent protease calpain. Immunostaining and Western blot analysis showed that both calpain and calcineurin are present at nerve terminals. Pharmacological inhibition of calpain with calpain inhibitors calpain inhibitor I, MDL-28170, and PD 150606, but not the control compound PD 145305, inhibit LFD both in the intact animals as shown by electromyograms and in dissected preparations as shown by intracellular recordings. These inhibitors probably act presynaptically as suggested by miniEPSP analysis. Calpain activity in CNS extract detected using a fluorimetric assay was modulated by calcium and calpain inhibitors. A cDNA encoding a partial calcineurin A sequence was obtained with RT-PCR using degenerate primers to a highly-conserved sequence in homologous genes from other species. Western blot analysis with an antibody against calcineurin A showed that calpain mediated proteolysis of calcineurin could occur in crayfish preparation in the presence of high Ca2+, but not in the presence of high Ca2+ and inhibitors of calpain. Inhibition of LFD by calpain inhibition causes rearrangement of the tubulin cytoskeleton at phasic terminals. High frequency depression (HFD) does not involve protein phosphorylation- or calpain-dependent mechanisms. This suggests that LFD might involve a specific pathway in which local Ca2+ signaling activates calpain and calcineurin at active zones and causes changes of tubulin cytoskeleton.In conclusion, calcineurin activated by presynaptic calpain may regulate LFD.

Reproduction and Development Platform



Repro: Orals

SIGNALING THROUGH FGFR2IIIC IS CRITICAL FOR MURINE EMBRYONIC STEM CELLS AND INDUCED PLURIPOTENT STEM CELLS COMMITMENT TO A LUNG EPITHELIAL CELL LINEAGE.

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The development of the lung is a complex process involving the cross talk between the mesenchymal and epithelial tissue layers. The importance of mesenchymal signaling in committing early endodermal cells into a functional epithelium has been well studied and suggests a large role for the FGF family of growth factors for directing the differentiation of the epithelium. Recent embryonic stem cell (ESC) studies have shown that in the appropriate microenvironment, these cells can differentiate into the progenitor cells of the target organ. While early studies have shown that lung mesenchyme has the capacity to differentiate ESC to an early lung cell lineage, it is still unknown the pathway this commitment is occurring through. The current study used early embryonic rat lung mesenchyme and combined it with ESC or iPSC that had been induced towards an endoderm lineage using activin A. ESC and iPSC cultured with mesenchyme expressed early markers of lung epithelium including TITF1 and pro-SFTPC. Electron microscopy demonstrated that the cells had the phenotypic characteristic of an early, albeit immature, distal lung cell phenotype. In order to determine the pathways involved in this commitment, truncated soluble receptors for the FGFR2 (sFGFR2) family were used. Mesenchyme- ESC or iPSC treated with sFGFR2iiib were still able to turn on expression TITF1 and pro-SFTPC, but lacked the tubule organization seen in non-treated controls. Treatment with sFGFR2iiic completely blocked expression of TITF1 and pro-SFTPC, suggesting a critical role for this pathway in commitment of endoderm induced ESC and iPSC to a lung cell phenotype.

PRO-INFLAMMATORY CYTOKINES AND CHEMOKINES INDUCED BY MECHANICAL STRETCH OF MYOMETRIAL CELLS CAN PROMOTE NEUTROPHIL INFILTRATION BY ENHANCED ADHESION AND TRANSENDOTHELIAL MIGRATION

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Current knowledge in labour mechanism is limited. Spontaneous labour at term is associated with increased cytokine production, cell adhesion molecule (CAM) expression and leukocyte invasion into the myometrium. We previously demonstrated an association between physiologic stretch and these inflammatory events in term rat myometrium. This coincided with the initiation of labour *in vivo*. We hypothesize that mechanical stretch induces human myometrial secretion of pro-inflammatory cytokines/chemokines that facilitate neutrophil transendothelial migration (TEM) into the myometrium. We cultured human myometrial SMC line (hTERT-HM) on flexible-bottomed collagen I-coated culture plates and applied static mechanical stretch for 24 hours (h). Stretch-conditioned media (SCM) and total RNA were collected and analyzed with multiplex human cytokine assays (Bio-Rad) and Real-Time PCR respectively. Next we examined the activation states of both primary human neutrophils and human uterine myometrial microvascular endothelial cells (UtMVEC-Myo) after stimulation with SCM. We used Real-Time PCR to analyze the mRNA expressions of neutrophil activation markers (CD11a, CD11b, CD44) as well as endothelial CAMs (E-selectin, ICAM-1, VCAM-1, PECAM-1). Adhesion and TEM assays were performed with UtMVEC-Myo to examine whether SCM promote the adhesion and TEM of primary human neutrophils. Levels of IL-6, IL-8, VEGF and GRO-α in SCM were significantly elevated by 24h stretch. Stimulation with SCM for 4 hours significantly increased endothelial E-selectin, ICAM-1 and VCAM-1

mRNA expressions, while stimulation with VEGF alone induced VCAM-1 expression only. SCM significantly increased neutrophil attachment to UtMVEC-Myo by 2.42-fold as compared to control media, while also enhancing neutrophil TEM. In a separate set of experiments we demonstrated that neutrophils transmigrated towards IL-8 and GRO-α stimuli in a dose-dependent manner. Altogether, these data indicate that VEGF acts as an endothelium activator, whereas IL-8 and GRO-α operate primarily as leukocyte recruiters. Moreover, SCM resulted in a more prominent endothelium activation and subsequent extravasation of neutrophils. Overall these results support our hypothesis that mechanical stretch can induce cytokine secretion capable of promoting peripheral leukocyte entry into the myometrium, which in turn, promotes a localized myometrial inflammation and the onset of labour.

DYNAMIC CHANGES IN MATERNAL DECIDUAL LEUKOCYTE POPULATIONS ACROSS FIRST AND SECOND TRIMESTERS OF HUMAN GESTATION

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One of the most crucial events of early pregnancy is remodeling of the uterine spiral arteries and the proper establishment of blood flow to the utero-placental interface. Part of this process involves the invasion of extravillous trophoblast and the concurrent recruitment of a large infiltrate of maternal leukocytes into the decidua, both of which are key to the remodeling process. At the time of infiltration, the maternal leukocytes are comprised primarily of uterine Natural Killer (uNK) cells and macrophages, and a very small number of dendritic cells, neutrophils, and T cells. However, these populations invariably change throughout pregnancy along with the constantly evolving needs of the fetus and the mother. Using immunohistochemistry and multi-colour flow cytometry, we have been able to characterize and define changes in leukocyte number and phenotype between the first and second trimesters, a critical preliminary step in identifying anomalies in leukocyte numbers in pathological pregnancies and potential functions of each subpopulation at varying stages of pregnancy. Briefly, a significant decrease in uNK cells, CD205+ dendritic cells, and HLA-DR+ expressing activated leukocytes was observed with increasing gestation. Proportions of total CD3+ T cells, CD4+ helper T cells and mature differentiated macrophages rose significantly from the first to second trimesters. Unexpectedly, our experiments also detected a large second trimester population of neutrophils significantly different from first trimester levels. Immunohistochemical staining further indicated that these neutrophils were localized only to decidua basalis and not parietalis. Interestingly, total leukocyte numbers did not change significantly between trimesters. Further experiments are in progress to investigate how specific decidual leukocyte subpopulations contribute to critical processes of pregnancy between the first and second trimesters, particularly the role of neutrophils in mid-pregnancy.

Repro: Posters

R1. LIPID HYDROPEROXIDE FORMATION FOLLOWING BIRTH REGULATES ALVEOLAR FORMATION AND PHYSIOLOGICAL LUNG CELL APOPTOSIS IN NEONATAL RATS

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Reactive oxygen species, including lipid hydroperoxides (LOOHs), play a critical role as second messengers in many physiological processes in the body. Their role in regulating alveologenesis, which occurs entirely postnatally in rodents, and physiological lung cell apoptosis is not completely understood. We hypothesized that the increase in arterial partial pressure of O_2 at birth (from approximately 20 to 70 mm Hg in the human) would induce low-level LOOH formation, which, in turn, would trigger the start of alveolar formation and regulate physiological lung cell apoptosis observed after birth. Diphenyl-phenyl diamine (DPPD), a LOOH-scavenger, was injected subcutaneously from days 1-6 of life in air-exposed neonatal rats. The effects of DPPD on alveolar formation and physiological lung cell apoptosis were assessed by lung morphometry, immunohistochemistry and Western blot analyses on day-7 samples. Add-back experiments were conducted using a prototypical LOOH, *t*-butyl hydroperoxide (*t*-BuOOH). Treatment with DPPD resulted in a significant increase in tissue fraction and mean linear intercept, significant reductions in small peripheral blood vessels and in both secondary crests/mm^2 and secondary crests/tissue ratio, as well as significant reductions in total BrdU-positive cells/mm^2, BrdU-positive secondary crests/mm^2 and BrdU-positive secondary crests/tissue ratio. Additionally, treatment with DPPD caused a significant reduction in estimated alveolar number. Decreased numbers of apoptotic cells, and decreased contents of the pro-apoptotic cleaved caspases-3 and -7 and cytoplasmic cytochrome *c* and an increase in anti-apoptotic Bcl-xl were found in lung homogenates treated with DPPD. Add-back experiments showed a dose-dependent reversal of the increase in tissue fraction in pups treated with both DPPD and *t*-BuOOH. In neonatal rats, LOOHs play a critical role in triggering postnatal secondary crest formation and resultant alveolar formation. LOOHs also regulate physiological apoptosis normally evident in the lung after birth as the alveolar-capillary barrier reduces in thickness.

R2. VEPH1 IS A NOVEL REGULATOR OF CANONICAL TGF-β SIGNALING IN OVARIAN CANCER CELLS

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Altered transforming growth factor-β (TGF-β) signaling is associated with the pathogenesis of epithelial ovarian cancer (EOC). Factors contributing to TGF-β resistance to growth suppression in EOC cells remain to be elucidated. A previous protein-protein interaction screen indicated interaction of FLJ12604, a variant of ventricular zone-expressed pleckstrin homology domain-containing protein 1 (VEPH1), with activin receptor-like kinase 5 (ALK5), suggesting VEPH1 may modulate TGF-β signaling. *VEPH1* was recently shown to be amplified in EOC, with correlated increased mRNA levels. To determine if and how VEPH1 regulates canonical TGF-β signaling in EOC cells. *VEPH1* transiently transfected cells were used to determine its effect on TGF-β signaling using reporter assays. SKOV3 cells stably expressing VEPH1 were generated and VEPH1 effect on TGF-β targets was examined by real-time RT-PCR or Western blot analysis. Gene expression profiling was performed to examine VEPH1 impact on TGF-β-induced responses. SMAD2 phosphorylation and nuclear accumulation were examined by Western blot analysis. Protein interactions were determined by co-immunoprecipitation. Coimmunoprecipitation confirmed VEPH1-ALK5 interaction. VEPH1 reduced TGF-β activation of SMAD2/3-dependent reporters and endogenous target gene expression. Profiling studies confirmed muted TGF-β transcriptional response. VEPH1 reduced nuclear SMAD2 and increased interaction of SMAD2 with activated ALK5. Our studies provide strong evidence of VEPH1 inhibition of canonical TGF-β signaling and support a model whereby VEPH1 impedes the release of SMAD2 from ALK5. Thus, VEPH1 up-regulation in EOC may contribute to preventing TGF-β-mediated tumor suppressor responses.

R3. EFFECTS OF VEPH1 ON FOXO-REGULATED SOD2 IN EPITHELIAL OVARIAN CANCER CELLS IN RESPONSE TO OXIDATIVE STRESS

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Inflammation associated with ovulation can result in cell signalling disruptions leading to ovarian malignant transformation in susceptible cells. The FoxO signalling cascade acts to prevent cells from accumulating DNA damage in response to oxidative stress by inducing the expression of the reactive oxygen detoxifying enzyme superoxide dismutase 2 (SOD2); dysregulation of this pathway may be an important event in ovarian carcinogenesis. Ventricular zone expressed PH domain protein (Veph1) may be a novel disruptor of FoxO signalling as its *Drosophila* orthologue Melted interferes with this cascade. We hypothesize that Veph1 decreases FoxO-mediated SOD2 expression and activity in response to oxidative stress. The ovarian cancer cell line SKOV3, which expresses low endogenous levels of Veph1, was stably transfected with a metallothionein inducible Veph1 expression construct (SKOV3-VE). SKOV3-VE and control (Mock) cells were exposed to oxidative stress induced by treatment with 200 μM of hydrogen peroxide (H_2O_2) for 0, 24, 48, and 72 hours. The expression and activity level of SOD2 was determined by western blot analysis and by measurement of superoxide radical dismutation, respectively. In addition, HEY and ES2, ovarian cancer cell lines which express high levels of endogenous Veph1, were stably transfected with a tetracyclin-inducible shRNA targeting Veph1 in order to further examine the effect of Veph1 expression. Interactions between Veph1 and FoxO signalling mediators were investigated using

coimmunoprecipitation. SKOV3-VE cells expressed decreased protein levels of SOD2 independent of H_2O_2 treatment as compared to Mock cells. Mock cells exhibit higher SOD2 activation in a time-dependent manner in response to H_2O_2 treatment than SKOV3-VE cells. Coimmunoprecipitation revealed an interaction between Veph1 and 14-3-3ε proteins, which mediate FoxO inhibition. Preliminary results indicate that Veph1 disrupts SOD2 regulation in response to oxidative stress in SKOV3 cells. Further validation will be conducted in HEY and ES2 cells.Veph1 may contribute to early events in ovarian carcinogenesis.

R4. CHARACTERIZATION OF HYPOTHALAMIC KISSPEPTIN CELL MODELS: CONTROL OF THE KISS-1 SYSTEM BY SEX STEROIDS

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Kisspeptin, a hypothalamic neuropeptide and product of the Kiss-1 gene, as well as its G protein-coupled receptor, GPR54, are essential for the initiation of puberty and maintenance of reproductive function throughout life. While an abundance of *in vivo* studies exist, due to the heterogeneity and inaccessibility of the hypothalamus, the molecular mechanisms underlying regulation of the Kiss-1 gene remain largely unknown. To address this concern, our lab has generated novel immortalized, clonal, murine cell lines derived from adult hypothalamic primary culture. Through the use of RT-PCR, immunocytochemistry and ELISA, we have established over twenty cell lines that exhibit endogenous kisspeptin synthesis. Two distinct populations of kisspeptin neurons have been identified within the hypothalamus: in the arcuate nucleus (ARC) and anteroventral periventricular nucleus (AVPV). These populations respond differentially to estrogen stimulation: kisspeptin neurons in the ARC are negatively regulated, while those in AVPV are positively regulated. Studies in our lab have shown that amongst our kisspeptin-expressing cell lines, the mHypoA-51 and mHypoA-63 models express ERα and ERβ, and are down-regulated by 10nM 17β-estradiol treatment, suggesting that they are representative of ARC kisspeptin neurons. Due to evidence indicating that kisspeptin neurons in the ARC co-express substance P, while those of AVPV co-express tyrosine hydroxylase (TH), we performed co-expression studies to further characterize these cell models. Presence of substance P mRNA was detected, while TH expression was absent in both mHypoA-51 and mHypoA-63, supporting their proposed identity as ARC kisspeptin neurons. Furthermore, ICC and EIA studies revealed that both models synthesize kisspeptin protein and basally secrete kisspeptin. RT-PCR has shown strong kisspeptin expression in another one of our cell models, mHypoA-55. Further screening confirmed expression of ERα and ERβ, substance P, but not TH, suggesting that mHypoA-55 is also representative of an ARC kisspeptin cell model. Preliminary studies with mHypoA-55 demonstrate induction of c-fos expression with a 2-hour 10nM 17β-estradiol treatment, indicating sensitivity of the cells to estrogen. We are currently examining the direct and dose-dependent effects of 17β-estradiol on Kiss-1 mRNA transcript levels in this cell model. Additionally, several of our kisspeptin-expressing cell models are being screened for various hypothalamic markers that are known to be co-localized with kisspeptin neurons in ARC and AVPV in order to better characterize. We intend to use our novel cell lines to investigate the regulation of kisspeptin synthesis in the hypothalamus.

R5. EFFECT OF *LACTOBACILLUS RHAMNOSUS* GR-1 (GR-1) ON CYTOKINES AND CHEMOKINES IN MATERNAL PLASMA, AMNIOTIC FLUID AND INTRA-UTERINE TISSUES OF PREGNANT CD-1 MICE.

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Probiotic *Lactobacillus* confers health benefits and GR-1 supernatant increases the anti-inflammatory cytokine interleukin (IL)-10 and decreases pro-inflammatory cytokine tumor necrosis factor (TNF)-α in human placental trophoblast cells. Studies were initiated to determine the effect of GR-1 on cytokine and chemokine secretion profiles in pregnant CD-1 mice. In two groups of pregnant mice, a daily dose of GR-1 at 10^8, 10^9 or 10^{10} colony forming units (cfu) or saline was orally administered for 7 days from gestational day (gd) 9-15. The first group was monitored until term (gd 20). The second group was sacrificed on gd 16. Twenty-one cytokines and chemokines in maternal plasma (MP), amniotic fluid (AF) and intra-uterine tissues (myometrium, decidua, placenta, fetal membrane) were measured using Bioplex. Oral administration of GR-1 did not alter the gestational length, but caused a significant increase in IL-6, IL-17, TNFα, and IL-10 in the MP at 10^9 cfu, and in the AF at a 10 fold higher dose (10^{10} cfu) except for IL-10. GR-1 (10^{10} cfu) significantly increased the chemokines KC and MIP-1β in the AF, but not in the MP. At 10^9 cfu, GR-1 significantly decreased TNFα, IL-10, MIP-1β, RANTES, and IL-3 in the myometrium. The above cytokines except RANTES, and IL-2, IL-5, IL-9 and IL-17 were significantly increased in the placenta. In the fetal membranes, GR-1 at 10^9 cfu significantly elevated IL-10, RANTES, and IL-3. There was no change in cytokines in the decidua. Th2/Th1 ratios were increased substantially by GR-1 (10^9 cfu) in the fetal membranes and the placenta, but were significantly decreased in the myometrium. We conclude that GR-1 administered orally to pregnant CD-1 mice alters the innate immunity, leading to differential cytokine and chemokine profiles in the MP, AF and intra-uterine tissues. Th2 dominance was observed in fetal membrane and placenta while Th1 dominance was observed in the myometrium.

R6. LUNG TISSUE ENGINEERING: GENERATION AND CHARACTERIZATION OF 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. The development of biomatrices that recapitulate the *in vivo* environment is a key component to driving differentiation of pluripotent cells into lung endoderm precursors. We investigate whether decellularized

cadaveric lungs with intact matrix composition, devoid of all cellular components, can promote the differentiation of ES cells into distal lung epithelial cells. Rat cadaveric lungs were decellularized by tracheal lavages and pulmonary perfusion using a range of physical, chemical, and enzymatic treatments. Histological staining, IF, electron microscopy, and tensile testing have confirmed decellularization and preservation of matrix proteins. Murine ESC were seeded onto scaffolds following endoderm induction using activin, and analysed for lung lineage marker expression. Seeded ES cells maintained FOXA2 expression and adopted an epithelial-like tubular organization. This demonstrates the ability of acellular lung scaffolds to support the adherence, proliferation, and potential differentiation of murine embryonic stem cells. Current studies are analyzing their potential as viable scaffolds for the unidirectional differentiation of human endoderm-induced ESC into distal lung epithelial cells.

R7. CHARACTERIZATION OF BOVINE OVIDUCTAL EPITHELIAL CELLS IN CULTURE AND INFLAMMATORY RESPONSE TO FOLLICULAR FLUID EXPOSURE.

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Evidence suggests that high-grade serous ovarian carcinoma (HGSC) originates from the distal fallopian tube epithelia (FTE). Ovulation is a micro-inflammatory event, of which a delayed resolution may lead to cellular transformation within the FTE. Our previous studies indicate that FTE from *BRCA1* mutation carriers exhibit a proinflammatory gene expression signature during the luteal phase, suggesting that delayed resolution of post-ovulatory inflammatory signalling may contribute to HGSC predisposition. To study mechanisms involved in this resolution, we sought to develop an *ex vivo* culture system using bovine oviductal epithelial (BOE) cells derived from abattoir specimens. **Objective:** To determine if BOE cells exposed to periovulatory follicular fluid (FF) *ex vivo* exhibit a proinflammatory response similar to that described for human FTE cells *in vivo*. Follicular Fluid was collected from consenting patients undergoing oocyte retrieval, and samples were analyzed for various cytokines via bioplex assay. Mechanically derived BOE cells were grown on collagen IV-coated transwells. Cells were characterized by immunohistochemistry and exposed to human FF or control medium for 24 hrs. RNA was extracted at various times up to 72 hrs after removal of FF and the impact on gene expression was determined by real time RT-PCR. Follicular fluid samples contain various cytokines, chemokines and growth factors, and does not impart a negative effect on viability of BOE cells. BOE cells grown on collagen IV exhibited epithelial morphology and stained for ciliated and secretory cell markers. Staining for vimentin was minimal. Consistent with human *in vivo* data, exposure of BOE cells to FF decreased DAB2 and increased IL8, which returned toward baseline levels following FF removal. BOE cells grown in primary culture offer a readily available system to explore mechanisms affecting the resolution of proinflammatory signalling in the FTE following ovulation. This work could indicate key events that predispose to HGSC.

R8. TRANSFORMING GROWTH FACTOR-β1 REGULATES MULTIDRUG RESISTANCE AT THE DEVELOPING BLOOD-BRAIN BARRIER

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P-glycoprotein (P-gp), a drug transporter responsible for mediating multidrug resistance at the developing blood-brain barrier, increases in activity during late gestation. The mechanism of this upregulation remains unclear. Astrocytes may have a prominent role in this upregulation as studies have shown that P-gp mRNA increases in brain endothelial cells (BECs) co-cultured with astrocytes. Transforming growth factor-β1 (TGF-β1) is a cytokine secreted by astrocytes during development and is critical in regulating cellular proliferation, differentiation, ECM production and apoptosis. Recent studies have shown the neuroprotective effects of TGF-β1. Very little work has been done on the neuroprotective effect of blood/peripheral- and astrocyte-derived TGF-β1 during fetal and neonatal brain development. In the present study, we hypothesized that TGF-β1 increases P-gp activity at the developing blood-brain barrier. BECs were isolated from postnatal day (PND) 14, gestational day (GD) 65 and 50 male guinea pigs. To assess the effect of peripheral TGF-β1, BECs were grown on 96-well cell culture plates. Varying doses of TGF-β1 (10^0-10^4 pg/ml) were added to the luminal side of BECs for 2, 4, 8 and 24 hours. Following TGF- β1 treatment, P-gp function was measured via calcein-acetoxymethyl ester assay. Cell viability was measured by trypan blue staining. Preliminary results demonstrate that TGF-β1 exposure to the luminal side of PND14 BECs elicited a significant increase in P-gp function at 2 ($P< 0.05$), 4 ($P< 0.05$), and 8 hours ($P< 0.05$). Peak responses were seen at 8 hours with approximately a 45% increase in P-gp function on BECs treated with 10^4 pg/ml of TGF-β1. At 24 hours of exposure, there was no significant change in P-gp function. Trypan blue staining indicated no significant cell death in BECs exposed to 10^3 or 10^4 pg/ml TGF-β1 for 24 hours. These preliminary results indicate that the effect of TGF-β1 exposure on the luminal side of PND14 BECs elicit a short-term increase in P-gp function. This data also suggests that TGF-β1 may mediate astrocyte-induced upregulation of P-gp function. Further experiments are being undertaken to investigate the role of astrocyte-derived (basolateral exposure) TGF-β1 in P-gp regulation. In addition, the role of TGF-β1 on P-gp function in the blood-brain barrier through late gestation will also be determined.

R9. EFFECTS OF PRENATAL SYNTHETIC GLUCOCORTICOID EXPOSURE ON HYPOTHALAMO-PITUITARY-ADRENAL (HPA) FUNCTION IN JUVENILLE OFFSPRING

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Synthetic glucocorticoids (sGCs) are commonly used to promote fetal lung maturation in women at risk of preterm labour.

However, animal models have demonstrated that prenatal sGC exposure modifies hypothalamic-pituitary-adrenal (HPA) function in first (F_1) and second (F_2) generation adult offspring. Little is known about the impact of sGC on HPA-function in juvenile animals. We hypothesised that prenatal sGC treatment results in substantial alterations in basal and activated HPA-function in juvenile F_1 offspring. Pregnant guinea pigs were treated with betamethasone (BETA; 1mg/kg) or saline (control) at gestational days 40/41, 50/51 and 60/61. Open field tests were conducted on post-natal days (PND) 19 and 24 with salivary cortisol sampling at 0, 30, 60 and 120 minutes, to assess HPA-responsiveness to challenge. Circadian variation in basal salivary cortisol was assessed at PND26, with samples taken at 0, 4, 8 and 24 hours. Salivary cortisol was measured using enzyme-linked immunosorbent assays. Cortisol levels increased significantly following exposure to the open field stressor, although response latency varied markedly amongst different combinations of sex and treatment. BETA females produced a greater peak cortisol response compared to controls at PND19 (30 minutes; $p < 0.05$). A similar relationship was identified in males at PND24. At PND26, BETA females exhibited an increase in cortisol production at 12pm ($p < 0.01$), with no increase in controls. In contrast, cortisol levels showed no circadian variation in BETA males, but were significantly elevated at 4pm in controls ($p < 0.05$). In both sexes, control animals showed no change in basal cortisol with advancing post-natal age. However, BETA females exhibited a significant reduction in basal cortisol at PND24 compared to PND19 ($p < 0.05$), whilst a comparable reduction was identified in BETA males at PND26 compared to PND19 ($p < 0.01$). Antenatal sGC exposure caused significant sex-specific alterations in basal and activated HPA-function in juvenile F_1 offspring. We are currently defining the molecular mechanisms which underlie the alterations in HPA-function following sGC exposure.

R10. SUPPRESSOR OF FUSED CONTROLS CELL DIFFERENTIATION IN THE CEREBELLUM VIA GLI3 REPRESSOR

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Suppressor of fused (*SuFu*) is a gene encoding an intracellular protein that regulates GLI activators and repressors, transcriptional effectors of the Sonic Hedgehog (SHH) signaling pathway. Importantly, SUFU stabilizes GLI activators and promotes processing of full-length GLI3 into its truncated repressor form (GLI3R) in the absence of SHH ligand. *SuFu* has been implicated as a tumor suppressor in medulloblastoma, cancer that arises from abnormal differentiation of granule cell precursors in the cerebellum. However, its role during cerebellar development is undefined due to the early embryonic lethality of *SuFu* germline knockout mice. Thus, we investigated the function of *SuFu in vivo* by generating mice with targeted *SuFu* deletion (*SuFu*-cko mice) in the mid-hindbrain, the origin of the cerebellum, using *Cre* recombinase driven by a *Hoxb7* promoter. *SuFu*-cko mice exhibited impaired motor coordination and severe cerebellar mispatterning. Loss of *SuFu* resulted in delayed differentiation of all major cerebellar cell types. Further, GLI3R protein was barely detectable in the absence of SUFU. Restoration of GLI3R in SUFU-deficient cerebella significantly rescued defective motor coordination, cerebellar mispatterning and abnormal cell differentiation, suggesting an important role for GLI3R in mediating SUFU functions. In order to determine the cell lineage-specific functions of SUFU, we deleted *SuFu* in granule cells (GC-*SuFu*-cko) and radial precursors (RP-*SuFu*-cko), respectively, using lineage-specific *Cre* recombinases. GC-*SuFu*-cko cerebella exhibited ectopic granule cells and delayed granule cell precursor differentiation, which were rescued by GLI3R expression. RP-specific deletion of *SuFu* resulted in delayed differentiation of all RP-derived cell lineages and severe cerebellar mispatterning that resembled the *Hoxb7-Cre* mutants. *SuFu*-deficient RPs were maintained in an undifferentiated state, resulting in delayed cell lineage specification. Restoration of GLI3R expression in RG-*SuFu*-cko cerebella significantly rescued abnormal cerebellar patterning and cell differentiation. Together, our data demonstrate that GLI3R-mediated functions of SUFU are required for cerebellar cell differentiation and morphogenesis.

R11. PULMONARY-SELECTIVE VASODILATION WITH NEBULIZED RHO-KINASE (ROCK) INHIBITOR IN NEONATAL RATS WITH CHRONIC PULMONARY HYPERTENSION

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Activation of Rho-associated protein kinase (ROCK) in smooth muscle is critical to sustained vasoconstriction and vascular remodeling in pulmonary hypertension (PHT). Systemic treatment with ROCK inhibitors (Y-27632 or Fasudil) reverse chronic PHT; the caveat being severe hypotension due to a lack of pulmonary selectivity. We hypothesized that inhalation of nebulized ROCK inhibitor (Fasudil) may confer pulmonary selectivity and thus avoid systemic effects. Rat pups were exposed to normobaric hypoxia (13% O2) from postnatal day (PND) 1 or were treated with bleomycin sulfate 1 mg/kg/d i.p. from PNDs 1-14. On PND 17 or 18, spontaneously breathing pups were placed in a custom plexiglass chamber (Buxco) and exposed to nebulized normal saline or Fasudil at one of three concentrations (150 ["low-dose"], 225 ["intermediate-dose"] or 300 mM ["high-dose"]) for 15 min using an Aeroneb Lab Micropump Nebulizer. Positive control pups were treated with systemic Y-27632 15 mg/kg i.p. At baseline, and 15 min post-treatment, pulmonary vascular resistance (PVR) was estimated by 2D echo/pulse wave Doppler and systemic blood pressure (BP) was recorded using a tail cuff Doppler device (n=6-8/gp). Lung and right ventricular (RV) free wall tissues (n=4/gp) were flash frozen within 30 min of treatment for Western blot analyses of ROCK activity (phospho-Thr850-MYPT-1). Compared to air-exposed or vehicle-treated controls, PVR and lung ROCK activity were significantly ($p<0.05$) increased by hypoxia or bleomycin. Increased PVR and lung ROCK activity were significantly ($p<0.05$) attenuated by all doses of nebulized Fasudil. Only high-dose (300 mM) Fasudil significantly ($p<0.05$) attenuated RV ROCK activity and decreased systemic blood pressure in both models. We conclude that

inhalation of nebulized fasudil can inhibit lung ROCK activity and normalize PVR without causing significant systemic effects.

R12. DIFFERENTIATION OF MOUSE AND HUMAN EMBBRYONIC STEM CELLS TO KIDNEY PRECURSORS THROUGH THE VARIATION OF GROWTH FACTOR COMBINATIONS, CONCENTRATIONS AND TEMPORAL GRADIENTS.

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Controlled differentiation of human and mouse embryonic stem cells lines have been used to produce cells of various lineages including kidney precursors. Our goal is to increase the efficiency of intermediate mesoderm differentiation so that we may direct these cells to the kidney lineage. Furthermore, co-culturing differentiated stem cells on a decellularized kidney matrix has been shown to support stem cell differentiation, therefore we are also interested in determining how to optimize and grow the embryonic stem cells with the long term goal of deriving a kidney. We hypothesize that pluripotent stem cells (ES or iPS) can efficiently be differentiated into kidney precursor cells through the addition of growth factors including members of the TGF-β superfamily, fibroblast growth factors and the Wnt family. We are currently growing human and mouse embryonic stem cell lines to differentiate into the mesoderm layer, the first stage of kidney development. We have grown these cells on collage type IV and gelatin coated plates over a course of 2 to 4 days with varied concentrations and combinations of BMP-4, Activin A and FGF2. We have been able to obtain brachyury (early mesoderm marker) positive cells in a number of conditions from both mouse and human stem cell lines with differing efficiency rates. We have also determined that the decellularized kidney matrix is able to support adult and fetal kidney cells. Once concentration and temporal conditions required for efficient mesoderm and intermediate differentiation are determined, we will be able to establish a protocol to direct ES cell differentiation towards a kidney cell fate, with our ultimate goal being the generation of an artificial kidney retaining the functional and structural integrity of a human kidney for clinical use.

R13. CENTRAL MECHANISMS FOR THE DIRECT INHIBITORY EFFECTS OF GONADOTROPIN-INHIBITORY HORMONE (GNIH) ON GONADOTROPIN-RELEASING HORMONE (GNRH) USING NOVEL HYPOTHALAMIC CELL MODELS

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Reproduction is coordinated by the actions of specific neuropeptides and peripheral hormones, all of which converge on the gonadotropin-releasing hormone (GnRH) neurons, which reside at the pinnacle of the hypothalamic-pituitary-gonadal (HPG) axis. Recently, a novel hypothalamic neuropeptide, gonadotropin-inhibitory hormone (GnIH), has emerged as a potent inhibitory modulator of neuroendocrine function. In mammals, GnIH appears to be localized in the dorsomedial hypothalamus and displays similar inhibitory functions as the avian form, though its distinct role in the HPG axis is not well established. To date, there is a paucity of studies focusing on the regulation of hypothalamic GnIH, as well as its potential direct regulation of GnRH neurons. We have generated immortalized, clonal, rodent cell lines derived from both embryonic and adult hypothalamic primary culture. Using semi-quantitative RT-PCR, we have classified a subset of cell lines that exhibit strong GnIH expression, as well as receptors for glucocorticoids (GR) and estrogen (ERβ and GPR30). We have also verified the presence of mammalian GnIH in two cell lines, rHypoE-19 and rHypoE-23, using an anti-RFRP antibody (generously provided by Dr. L.J. Kriegsfeld, UC Berkeley). In a newly established cell model of GnRH neurons, the mHypoA-GnRH/GFP, we confirmed the presence of the GnIH receptor, GPR147. These neurons were immortalized and FAC-Sorted from an adult GnRH-GFP mouse to generate a non-clonal cell line representative of the entire GnRH neuronal population. Using real time RT-PCR we analyzed the dose-dependent and direct effects of GnIH on GnRH mRNA expression. We have demonstrated that GnIH treatment (100 nM) directly represses GnRH mRNA expression by approximately 40% at 1 and 4 hours ($P<0.05$). Current studies using the GPR147 antagonist, RF9, and Western blot analysis are being used to delineate the direct GnIH/GPR147-mediated mechanisms controlling GnRH transcription. Preliminary evidence suggests that RF9 can attenuate the inhibitory actions of GnIH on GnRH expression. Future studies will explore the effects of GnIH on GnRH secretion, through both direct GnIH treatments and co-culture experiments. We anticipate that these novel hypothalamic GnIH cell models can be used to further define the cellular mechanisms by which GnIH input and signaling mediates mammalian reproduction through the modulation of GnRH.

R14. ALTERED MECHANISMS OF ACID SPHINGOMYELINASE REGULATION AND PROCESSING IN PREECLAMPSIA

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Sphingolipids act as bioactive mediators in several pathophysiological processes by regulating cell fate. Ceramides (CERs) are key effectors in pathways initiated by diverse stress stimuli. CER metabolism is controlled via the action of specific enzymes. Acid SphingoMyelinase (ASM) causes sphingomyelin hydrolysis and subsequent CERs generation. ASM is synthesized as a precursor in the endoplasmic reticulum (ER) with 6 N-linked oligosaccharide chains which are essential for ASM trafficking to the lysosomes where it is activated. The objective of this study was to examine CERs and ASM expression, function and processing in placentae from preeclamptic (PE) and normotensive age-matched control (AMC) pregnancies. Protein and mRNA expression levels of ASM were assessed by WB analysis and immunofluorescence (IF) in AMC and PE placentae using antibodies against ASM and calreticulin, a ER resident protein. ASM enzyme activity was evaluated using an fluorogenic enzyme coupled assay. Human villous explants and choriocarcinoma JEG-3 cells were treated with sodium nitropursside (SNP, a nitric oxide donor) or kept at either 3% or 20% O_2 and ASM post-

translational modification was analyzed using the glycosylation inhibitors peptide-N4-aspargine amidase F (PNGaseF), tunicamycin and/or concanavalin A. Lipid analysis in normal and PE placental tissue and in SNP-treated cells was performed using HPLC linked to mass spectometry (MS/MS). MS/MS revealed a significant increase in CERs and SM levels in PE relative to preterm (PTC) and term controls (TC) and this associated with an increased in ASM precursor protein expression. SNP increased CERs and both active ASM and its precursor levels in JEG-3 cells and explants. Decrease ASM enzyme activity was detected in PE and SNP treated explants and JEG-3 cells. Furthermore, treatment with PNGaseF and/or concanavalin A indicated that ASM undergoes glycosylation and that this post-translational modification events was disrupted in PE. IF analysis in section from PE and PTC placentae showed co-localization of ASM with calreticulin. Similarly, SNP caused ASM accumulated in the ER while its signal was absent in the lysosomes. In conclusion, altered ASM expression and processing in PE, due to oxidative stress, is responsible for CERs accumulation that in turn may contribute to increased cell death typical of this disorder.

R15. TROGOCYTOSIS PREVENTS REJECTION OF DONOR CELLS IN BONE MARROW TRANSPLANTATION

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Graft rejection has always been a concern for patients receiving allogeneic hematopoietic cells transplantation (AHCT) after reduced intensity conditioning (RIC) regimens. Although full myeloablative conditioning will decrease the risk of rejection, this conditioning regimen is not suitable for older patients, high risk patients with co-morbidities, or patients with non-malignant diseases. The development of immuno-compromised mouse models has enabled us to investigate the role of individual lymphocyte population in graft rejection. Using T and B cell-deficient NOD/SCID mice as our host, we identified that 100% of our surviving donor cells had acquired host's major histocompatibility complex (MHC) class I molecules through trogocytosis. We hypothesize that the presence of host MHC class I proteins on donor cells disguises them as 'self' and allows them to escape immunological attack from host's natural killer (NK) cells. Using T, B, and NK cell-deficient NOD/SCID/gamma (NSG) mice as hosts, we observed that not all engrafted donor cells were positive for host MHC class I proteins. The involvement of all surviving donor cells in trogocytosis in the NOD/SCID mice suggests that the presence of NK cells exerts a selective pressure for cells with host MHC class I proteins. According to the 'missing self' hypothesis, it is advantageous for donor cells to have host MHC class I proteins as these proteins can inhibit NK cell-mediated cell lysis through interaction their inhibition receptors. A better understanding of trogocytosis in the bone marrow transplantation setting, and how this affects donor cells survival in the presence and absence of NK cells can help us design RIC regimens that promote donor cell engraftment while decreasing irradiation dosage and reducing drug use.

R16. CHARACTERIZATION OF *IN VITRO* P-GP TRANSPORT IN HUMAN PLACENTAL TISSUE CULTURE

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Acute and chronic infections are common during pregnancy and there is evidence that infection can alter the activity of drug transporters, such as P-glycoprotein (P-gp). Specifically, studies have shown that cytokines inhibit P-gp mediated drug efflux activity in liver cells. However, knowledge of the relationship between infection and drug transporter activity and expression in the placenta is limited. The objective of this study was to characterize P-gp transport in the human placenta using an *in vitro* culture model. The establishment of this model system will provide a means to study transporter function and expression under various treatment conditions, such as infection. Term placentae were collected within 30 min of delivery from women with healthy pregnancies at Mount Sinai Hospital. Placental tissue was biopsied (8 mm^3) for measurement of P-gp activity and the remainder tissue was cultured for a 6-day period. Placental tissue explants were cultured at 37 °C (21% O_2, 5% CO_2). Culture medium was collected and replaced every 24h across a 6-day period. Lactate dehydrogenase (LDH) and human chorionic gonadotropin (hCG) released into the culture medium was measured to assess explant viability and syncytiotrophoblast function. P-gp activity was measured as accumulation of 1.5uM digoxin (a P-gp specific substrate; ^{3}H-digoxin was used as a tracer) at 10, 30, 60, 120 and 180 min. P-gp activity was measured in fresh tissue on day 0 and after 6 days of culture, to assess whether explants express higher levels of P-gp and activity before or after the culture period. There was an increase in digoxin (P-gp substrate) in fresh placental fragments over time, with a plateau phase reached at 120 min. Preliminary results (n=3) indicate a decreased rate of digoxin accumulation (i.e. increased P-gp activity) after 6 days of culture compared to fresh placental tissue (day 0). Cultured explants appear to have increased P-gp activity after a 6-day culture period. This *in vitro* placental explant model provides a means to measure changes in P-gp function, owing to the increase of P-gp activity on day 6 of culture. Furthermore, different treatments, such at cytokines to mimic infection, may be used to test their effects on P-gp function and expression. This is important; as changes in P-gp mediated placental drug function may have adverse effects on the developing fetus.

R17. TRANSGENERATIONAL EFFECTS OF ANTENATAL SYNTHETIC GLUCOCORTICOID TREATMENT ON LEARNING AND MEMORY

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Approximately 10% of pregnant women are at risk of preterm delivery. The majority of these women receive treatment with synthetic glucocorticoids (sGCs) to reduce the risk of infant respiratory distress syndrome. We have shown that prenatal sGC exposure alters stress responsiveness and locomotor activity in first (F_1) and second (F_2) generation offspring. Increased locomotor activity in F_1 offspring is associated with altered hippocampal NMDA receptor expression and hippocampal long-

term. In the present study, we hypothesized that maternal exposure to sGC results in impaired learning and memory in F_1 and F_2 offspring. Pregnant guinea pigs (F_0; n=8-10/gp) were subcutaneously injected with betamethasone (BETA; 1mg/kg) or vehicle (VEH; saline) on gestational days (gd) 40/41, 50/51 and 60/61. Adult F_1 female offspring from each group (n=7-8/gp) were mated with control males. F_1 and F_2 offspring underwent behavioral testing in a Morris Water Maze (a test for learning and memory) on postnatal days 35 (juvenile) and 70 (adult). Latency to find a hidden platform, retention of platform location and search strategy were analyzed. All groups effectively learnt the location of the hidden platform. However, there was no effect of prenatal (F_0) sGC exposure on latency to find the platform in juvenile or adult offspring in either generation. There were also no significant differences in memory of the platform location (probe trial) between any of the groups. However, both juvenile and adult female F_1 offspring whose mothers received sGCs during pregnancy used a different strategy to search for the platform's location during the probe trial ($p<0.05$), indicating a greater ability to adapt to changing conditions (i.e. removal of the platform during the probe trial). Together, these results suggest that the processes of memory and learning are resilient to the effects of prenatal exposure to excess glucocorticoids, while there are changes in how knowledge is applied

R18. MURINE PLACENTAL SYSTEM A EXPRESSION: DEVELOPMENT AND EFFECTS OF SYNTHETIC GLUCOCORTICOID TREATMENT.

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Synthetic glucocorticoids (sGCs), which are administered to women in threatened preterm labour, differentially regulate the system A amino acid transporter *in vitro*. Recently, we have reported that murine placental system A transport dramatically increases over the second half of gestation. However, sGC treatment in mid-gestation reduced system A transplacental transport at term. The three system A transporter proteins are encoded by *Slc38a1*, *Slc38a2* and *Slc38a4* genes. The molecular mechanisms underlying development of, and sGC induced alterations in, system A activity are not known. We hypothesized that *Slc38a* gene expression increases across gestation from embryonic day (E)12.5 to E18.5 and is down-regulated by maternal sGC treatment. In untreated C57BL/6 mice, mRNA expression was examined in placental tissue obtained from E12.5, E15.5 and E18.5 (term ~E19.5). Placental tissue was also obtained from pregnant dams treated with dexamethasone (0.1mg/kg) or saline on E13.5 and E14.5 to assess short-term (E15.5) and longer-term (E18.5) consequences on system A mRNA expression. Placental *Slc38a1*, *Slc38a2* and *Slc38a4* expression were measured by qRT-PCR. System A gene expression of *Slc38a1*, *Slc38a2*, and *Slc38a4* mRNA increased from E12.5 to E18.5 (*$p<0.05$; n=5-7 dams/group) in placentas from male and female fetuses; consistent with the increase in system A activity. No sex-specific differences in mRNA expression occurred across gestation. While we have shown sGC treatment to decrease system A activity at term, sGC treatment did not affect placental *Slc38a1*, *Slc38a2* and *Slc38a4* gene expression at E15.5 or E18.5 (n=8 dams/group). System A mRNA and activity increase across the second half of gestation to meet the increase nutrient demands of the fetus during this time. In addition, the sGC induced reduction in system A activity that we have reported at term is not mediated by alterations in system A mRNA expression. In this context, it is possible that post-transcriptional and/or post-translational modifications may mediate the reduction in system A activity.

R19. GLUCOCORTICOIDS (GCS) INCREASE MULTIDRUG RESISTANCE IN THE DEVELOPING BLOOD-BRAIN-BARRIER (BBB)

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P-glycoprotein (P-gp) protects the fetal brain from a wide range of xenobiotics and regulates the passage of endogenous compounds. P-gp levels in the early fetal brain are low, but dramatically increase near term. This increase is concurrent with the late gestation rise in endogenous GCs. GCs potently stimulate P-gp, and fetal brain endothelial cells (BECs) become more sensitive to GCs in late gestation. However, whether the endogenous GC surge is responsible for the rise in BBB P-gp is unknown. Using BEC cultures derived from fetuses exposed to synthetic GCs (sGCs) *in utero*, we investigated the regulatory effects of sGCs on P-gp function and BEC responsiveness to GCs. We hypothesized that prenatal sGC exposure will enhance P-gp function – mimicking the endogenous GC surge. Brain microvessels (BMVs) and BECs were isolated from gestational day (GD) 50 fetuses exposed *in utero* to dexamethasone (sGC; 1 mg/kg), or vehicle (VEH) on GD48/49. Confluent BECs were treated with 10^{-8}-10^{-5} M of cortisol, dexamethasone or aldosterone for 2-24h, and P-gp function was assessed (1 μM calcein-AM). P-gp protein was measured in BMVs. Treatment of VEH-exposed GD50 BECs with cortisol resulted in a trend of increased P-gp activity, but this was not significant ($P>0.05$). However, GD50 BECs derived from sGC-exposed fetuses displayed a significant dose-dependent increase in P-gp function when treated with cortisol (~40%; $P<0.01$). Treatment of BECs from both VEH and sGC-exposed fetuses with dexamethasone resulted in increased P-gp function (~40%; $P<0.01$), while aldosterone treatment had no effect ($P>0.05$). Prenatal sGC exposure increased in BMV P-gp protein, 2-fold ($P<0.05$). GCs are centrally involved in the regulation of multidrug resistance at the developing BBB. Exposure to sGCs prior to the endogenous GC surge matures BEC response to GCs – so that the response mimics that seen in near-term BECs (experienced endogenous GC surge). Mothers at risk of preterm labor are administered sGC to mature the fetal lungs. Our data suggests that this treatment prematurely increases fetal BBB multidrug resistance expression, potentially altering fetal brain protection against xenobiotics, but also the passage of endogenous compounds important for development.

E.D.R.G. Platform



E.D.R.G.: Orals

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

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Glucagon-like peptide-2 (GLP-2) is an intestinal L cell hormone released in response to nutrient ingestion that enhances gut barrier function. We have previously demonstrated that the actions of GLP-2 on intestinal proliferation are indirect and mediated through the actions of another growth factor, insulin-like growth factor-1 (IGF-1), on its receptor in the intestinal epithelium (ieIGF-1R). Since IGF-1 is also a known modulator of intestinal barrier function, we hypothesize that the actions of GLP-2 to modulate intestinal permeability also signal through activation of the ieIGF-1R. The ieIGF-1R was specifically targeted using inducible ieIGF-1R$^{-/-}$ mice. *In vivo* assessment of paracellular intestinal permeability using oral fluorescein isothiocyanate dextran 4000 (FD4) showed that vehicle-treated control animals had plasma FD4 levels of 1.4±0.3 ug/ml, at both 1.5 and 4h. Ten-day treatment of mice with Gly^{2}GLP-2, a potent GLP-2 analogue, significantly reduced intestinal permeability in control mice at 1.5h by 53% ($p<0.05$). However, GLP-2 did not lower gut permeability in the ieIGF-1R$^{-/-}$ animals. Moreover, Ussing chamber evaluation of jejunal tissue showed that resistance was elevated by 119% in control animals treated with GLP-2, as compared to those without treatment. This GLP-2-mediated elevation in jejunal resistance was reduced by 41% in knockout animals. At the molecular level, GLP-2 upregulated paracellular tight junctional protein ZO-1 expression by 206% in control mice, and this effect was abrogated in ieIGF-1R$^{-/-}$ mice. The GLP-2-mediated upregulation of ZO-1 was reflected by more intense immunofluorescent staining along the apical surface of intestinal villi in control animals. In contrast, GLP-2-treated knockout animals exhibit intracellular, as opposed to membranous, localization of ZO-1. Collectively, GLP-2-mediated enhancement of barrier function is reduced in the absence of the ieIGF-1R, and this can be attributed to changes in the tight junctional protein ZO-1, through lowered expression and disruption of subcellular localization.

P21-ACTIVATED PROTEIN KINASE 1 (PAK1) MEDIATES THE CROSSTALK BETWEEN INSULIN AND β-CATENIN ON PROGLUCAGON GENE EXPRESSION AND ITS ABLATION AFFECTS GLUCOSE HOMEOSTASIS

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The proglucagon gene (*gcg*) encodes several important hormones that regulate blood glucose homeostasis and satiety, including GLP-1 and GLP-2 in gut and brain, as well as glucagon in pancreas. *Gcg* expression is controlled by numerous cell signaling molecules/pathways in cell-type specific manners in response to nutritional/hormonal stimuli. We have identified previously that the Wnt signaling pathway effectors β-catenin (β-cat) and TCF7L2 are important for gut *gcg* expression, by mediating the stimulatory effects of cAMP and insulin. In this study, we hypothesized that 1) Pak1 is a novel mediator of the crosstalk between insulin and Wnt signaling in regulating *gcg* expression 2) Pak1 ablation perturbs glucose homeostasis. First, we assessed

the role of Pak1 in insulin-stimulated *gcg* expression and β-cat activation (S675 phosphorylation) in the intestinal L cell line GLUTag. Insulin stimulated Pak1 activation, associated with increased *gcg* mRNA expression and promoter activity. This stimulation was attenuated by the chemical inhibitor IPA3 or dominant negative Pak1. Concomitantly, insulin and cAMP-promoting agents activated β-cat S675 phosphorylation, and the activation was attenuated by IPA3 or PKA inhibition, respectively. Next, we investigated the role of Pak1 in glucose homeostasis using $Pak1^{-/-}$ mice. These mice exhibited impaired oral glucose tolerance and pyruvate tolerance tests. They had noticeably reduced gut *gcg* expression, accompanied with a trend of lower circulating levels of active GLP-1. In addition, brainstem *gcg* level was drastically reduced in $Pak1^{-/-}$ mice, and insulin-stimulated β-cat S675 phosphorylation was abolished in the brain neurons of $Pak1^{-/-}$ mice. In summary, we show here that the insulin-Pak1-β-cat axis is important for regulating gut and brain *gcg* expression, and Pak1 ablation impairs glucose homeostasis *in vivo*.

EFFECTS OF INSULIN RESISTANCE ON LEPTIN MODULATION OF HYPOTHALAMIC NEURONS

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The development of resistance to peripheral signals, insulin and leptin, is a critical problem associated with the failure of central mechanisms governing energy balance. However, the precise molecular mechanisms underlying neuronal insulin and leptin resistance remain unclear due to the lack of appropriate cell models. To this end, our laboratory has generated an array of immortalized hypothalamic cell lines (1). Using RT-PCR, we have identified a rat, embryonic cell-line, rHypoE-19, that endogenously expresses AgRP, urocortin, long-form leptin receptor (Ob-Rb), insulin receptor (IR), as well as key signaling molecules, IRS-1, IRS-2, and SOCS3. We hypothesize that insulin resistance at the level of the individual hypothalamic neuron can directly hinder neuronal leptin sensitivity. Using real-time RT-PCR we have demonstrated that 10 nM leptin and insulin treatment regulates AgRP, IR, PC1, SOCS3, and IRS-1 transcript levels at 1, 8 or 24 hours following treatment. Furthermore, we have established that insulin regulates these neurons through the insulin receptor substrate, phosphatidylinositol-3-kinase (PI3K) and the mitogen-activated protein kinase (MAPK) signaling pathways. We have also developed a model of neuronal insulin resistance via exposure of neurons to 100 nM insulin over 24 hours (2). We next confirmed that leptin signaling occurs through the janus tyrosine kinase (JAK)-signal transducer and activator of transcription (STAT), as well as the PI3K signal transduction pathways in the rHypoE-19 neurons. Interestingly, we have found that leptin signaling is significantly attenuated in the context of insulin resistance by analyzing the effects of leptin on the phosphorylation status of key signaling molecules prior to and following the induction of insulin resistance. In accordance with this, we have shown that leptins transcriptional regulation of the rHypoE-19 neurons is also attenuated following induction of insulin resistance. Ultimately, we aim to gain a more comprehensive understanding of the role of insulin in leptin-induced modulation of hypothalamic neurons. Investigation of the molecular mechanisms underlying leptin and insulin modulation of each others actions will lends itself to an improved understanding of the complex biological processes that underlie cellular hormone resistance at the neuronal level associated with obesity and type 2 diabetes.

E.D.R.G.: Posters

E1. AUTOPHAGY REGULATES EXPRESSION OF THE RISC COMPONENT AGO2 A CRITICAL REGULATOR OF THE MIRNA SILENCING PATHWAY

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Genome-wide association studies have repeatedly associated a variant of the autophagy dependent gene ATG16L1 with Crohn's disease (CD), thus implicating autophagy, a novel and unexpected pathway, in the development of inflammation. The functional relevance of the ATG16L1-CD variant is unclear, as it was shown to be unnecessary for canonical autophagy, and whether it affects bacterial mediated autophagy remains controversial. We hypothesized that the ATG16L1-CD variant is involved in a different process that contributes to persistent inflammation, the miRNA silencing pathway. MiRNA are short non-coding RNA that bind to target mRNA to repress their translation and promote their degradation. Emerging data suggests that altered regulation of pro- and anti-inflammatory genes by miRNA is involved in the pathogenesis of IBD. In order to exert their function, miRNA are loaded onto an RNA induced silencing complex (RISC). AGO2 is a critical component of the RISC complex and the only Ago protein with Slicer activity which results in the generation of unique mature miRNA. RISC formation and turnover occurs on endosomal membranes. Since autophagy and endosomal membranes are closely related, we hypothesized that autophagy and the ATG16L1-CD variant affect RISC components, hence modulating miRNA silencing. In order to test our hypothesis we performed immunoblotting assays to compare the expression of RISC components in control cells or cells with increased or disrupted autophagy using bafilomycin, knockdown of ATG dependent genes or treatment with the *H. pylori* vacuolating cytotoxin (VacA) which disrupts autophagy. We determined the colocalization of autophagic vacuoles with RISC components using confocal microscopy. Our data show increased AGO2 expression in epithelial cells in which autophagy is disrupted by knocking-down the expression of autophagy proteins atg16, atg5 and atg12, as well as treatment with VacA in comparison with control cells. Increased AGO2 expression was also detected by confocal imaging of cells with disrupted autophagy. In addition, human peripheral blood mononuclear cells also show increased AGO2 when autophagy was disrupted. Taken together, these results suggest that autophagy is involved in degradation of the critical RISC component AGO2 in both epithelial and myeloid cells. We propose a compelling mechanism by which autophagy may affect miRNA expression and is involved in the etiology of CD.

E2. PROGLUCAGON GENE EXPRESSION IS REGULATED BY INSULIN, LEPTIN AND cAMP IN MOUSE EMBRYONIC AND ADULT HYPOTHALAMIC NEURONAL CELLS.

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Proglucagon-derived peptides play important roles in energy homeostasis and appetite regulation. The proglucagon gene is expressed in islet α-cells and intestinal L-cells, as well as a limited number of hypothalamic neurons. At present, the regulation of hypothalamic proglucagon expression by insulin, leptin and cAMP, the key regulators of food intake and energy balance, remains unclear. The lack of knowledge is due to inaccessibility to the hypothalamic proglucagon neurons. Using mHypoE-39 and mHypoE-20/2 cell lines, derived from embryonic mouse hypothalamus, and mHypoA-2/10 neuronal cells, derived from adult mouse hypothalamus, we studied the mechanisms involved in the direct regulation of proglucagon gene expression by insulin, leptin and cAMP. We determined that insulin, in an Akt-dependent manner, significantly induced proglucagon mRNA expression by 70% in adult mHypoA-2/10 cells, while significantly suppressing it by 45% in embryonic mHypoE-39 cells. We further found that leptin also regulated proglucagon mRNA expression in these cell models via the JAK/STAT pathway. Leptin caused an initial increase by 66% and 43% at 1 h followed by a decrease by 45% and 34% at 12 h time points in mHypoA-2/10 and mHypoE-39 cells, respectively. Further, cAMP activation by forskolin upregulated proglucagon expression by 87% at 4h time point in mHypoE-39 cells, and it increased proglucagon gene expression through the Epac activation in mHypoE-20/2 cells. Transient transfection analysis determined that specific proglucagon promoter regions were regulated by cAMP in the embryonic cell lines, whereas an RNA stability assay demonstrated that leptin and insulin increase proglucagon mRNA stability in the adult cell line. These findings suggest that insulin, leptin and cAMP act directly on specific hypothalamic neurons to regulate proglucagon gene expression. The proglucagon-derived peptides are key regulators of feeding behavior, thus a better understanding of the regulation of hypothalamic proglucagon is important to further expand our current knowledge of feeding circuits.

THE ROLE OF R-SPONDIN 1 IN β-CELL NEOGENESIS

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Studies in rodents have demonstrated that the β-catenin-dependent canonical (c)Wnt pathway is involved in pancreatic development, islet function, and insulin production and secretion. We have recently reported that a new member of the cWnt signaling pathway, roof plate-specific spondin-1 (Rspo1), is expressed in murine islets and the murine MIN6 and βTC β-cell lines. Although Rspo1 increases β-cell proliferation and survival, as well as insulin secretion in these in vitro models, we also found that Rspo1 knockout (KO) mice have increased β-cell mass in association with a marked increase in the number of insulin+ ductal cells in the pancreas, suggestive of increased β-cell neogenesis. However, these studies were performed in basal, non-stressed conditions, and the role of Rspo1 during metabolic challenge is not known. We therefore hypothesized that Rspo1 inhibits β-cell neogenesis in streptozotocin-induced diabetes, both acutely (6-days; n=9-11for WT and KO) and chronically (32-days; n=11-16 for WT and KO) after diabetes induction. Co-immunohistochemistry for insulin and Ki67 or caspase-3 revealed that, although KO and age- and sex-matched wildtype littermates had similar levels of proliferative insulin+ cells, KO mice

demonstrated a reduction in β-cell apoptosis at both 6- ($p<0.05$) and 32- ($p=0.05$) days. Importantly, KO mice had a greater number of insulin+ ductal cells at both time points ($p<0.05$), and co-immunofluorescence for insulin and the duct cell markers cytokeratin-19 and carbonic anhydrase II further revealed the presence of insulin+ cells within ductal cells, thus indicating increased neogenesis in the absence of Rspo1. Consistent with this finding, qRT-PCR showed that KO mice also had increased pancreatic pro-insulin2 ($p<0.05$) at day 6. Although there was no difference in β-cell mass at both time points, KO mice demonstrated better glycemic control after an oral glucose tolerance challenge ($p<0.05$), suggesting a functional consequence to the increased neogenesis. Together, these findings indicate an important role for Rspo1 as a negative regulator of both β-cell survival and neogenesis in β-cell adaptation in a model of Type 1 diabetes.

THE SIRT1 ACTIVATOR RESVERATROL PREVENTS FAT-INDUCED B-CELL DYSFUNCTION IN VIVO

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Over the last decade, sirtuins and SIRT1 in particular, have been identified as key regulators of nutrients and metabolism. Within the β-cell, there is evidence that SIRT1 plays a beneficial role on insulin secretion. It has also been established that excess circulating fat as seen in obesity can be detrimental to β-cell function ("β-cell lipotoxicity"), an effect that may involve decreased SIRT1 activity. Consequently, it would seem logical that SIRT1 activation may have a beneficial role on β-cell function in conditions of nutrient excess. Here we attempted to mitigate lipotoxicity induced β-cell dysfunction using a pharmacological model of SIRT1 activation. Female Wistar rats (n= 4-8 per group) were infused intravenously for 48h with 1) Saline (SAL, control), 2) Oleate (OLE) at 1.4μmol/min to elevate plasma FFA by 50%, 3) Oleate + the SIRT1 activator Resveratrol (RSV, 0.025mg/kg.min) and 4) RSV alone (0.025mg/kg.min). The infusion period was followed by assessment of β-cell function by hyperglycemic clamp. OLE infusion resulted in decreased plasma insulin and c-peptide levels in response to the rise in glucose that were significantly decreased (>50%) compared to SAL control infusion ($p<0.05$). In the group coinfused with OLE+RSV, complete prevention of the oleate-induced decrease in insulin and c-peptide was seen compared to the SAL control infusion. No significant difference was seen between SAL and RSV infusion alone. These results indicate that RSV prevents the β-cell dysfunction caused by 48h OLE infusion, an effect that may be SIRT1 mediated.

E5. ANALYSIS OF POTENTIAL CANDIDATE COMPOUNDS THAT MAY ALLEVIATE CELLULAR INSULIN RESISTANCE IN IMMORTALIZED RHYPOE-19 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). Insulin signaling, at the level of the hypothalamus, is crucial to maintain glucose and energy homeostasis. Most cases of T2D are associated with insulin resistance, a condition characterized by a reduced cellular response to insulin. AMPK is a key enzyme involved in regulating energy homeostasis in insulin sensitive tissues, such as the hypothalamus, liver and β-cells, and is activated by an increase in the AMP/ATP ratio. In insulin sensitive tissue the activation of AMPK leads to the inhibition of mammalian target of rapamycin (mTOR), a downstream effector of the PI3K/Akt pathway. This subsequently prevents the activation of S6 kinase (S6K), which in turn removes the inhibition of insulin substrate receptor-1 (IRS-1). There is a growing body of evidence suggesting that 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR), an AMP mimetic, can attenuate insulin resistance within the liver, adipocytes, and β-cells by increasing the phosphorylation of AMPK. However, due to the complexity of the hypothalamus and lack of *in vitro* cell models, the effects of AMPK activating compounds on central insulin resistance are not well understood. We have generated a novel immortalized clonal hypothalamic neuronal cell-line, rHypoE-19, which will be used to investigate the effects of AMPK modulators, such as AICAR, metformin, compound C and C75 on cellular insulin resistance. We have created a model of cellular insulin resistance within the rHypoE-19 cell-line. Pre-treatment of the rHypoE-19 cells with vehicle (PBS) or 10 nM insulin for 24 hours induced phosphorylation of Akt 15 minutes following a 10 nM insulin re-challenge. However, we found that insulin signaling was attenuated with 100 nM and 1 μM insulin pre-treatment upon re-challenge. Using real-time RT-PCR and Western blot analysis our current goal is focused on investigating the effects of AMPK modulators on cellular insulin resistance in the rHypoE-19 cell model. Uncovering the pathways involved in central insulin resistance and determining how AMPK, an important energy-mediating enzyme, regulates these pathways will further our understanding of the intricate regulation of central energy homeostasis.

E6. DEVELOPMENT OF A NOVEL ZEBRAFISH SYSTEM TO SCREEN FOR POTENTIAL ANTI-DIABETIC DRUGS

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Phosphoenolpyruvate carboxykinase (PEPCK) gene is an excellent potential target for the screening of anti-diabetic drugs because it produces a rate limiting gluconeogenic enzyme, playing an important role in endogenous glucose production and metabolism. It has been shown to be a sensitive and stable marker for blood glucose levels because it is highly regulated in glucose metabolism. Promoter polymorphism at cAMP response element (CRE) has been found to be associated with the development of Type 2 Diabetes Mellitus in humans. PEPCK gene is highly conserved among humans, mice and zebrafish. PEPCK expression levels can serve as a marker for ant-diabetic drug efficacy in zebrafish. Compounds down-regulating PEPCK expression may have anti-diabetic efficacy and could potentially be developed as anti-diabetic drugs. The generation of novel fluorescent zebrafish reporter strains that can be used for screening anti-diabetic drugs in zebrafish embryos to target PEPCK gene. *In silico* analysis of a 4kb zebrafish PEPCK promoter sequence identified conserved transcription factors such as CRE.

PEPCK:Enhanced green fluorescent protein (EGFP) reporter lines of 0.8kb and 3.6kb in length are made via microinjection. EGFP expression pattern of the generated reporter transgenic lines are compared to the endogenous gene expression in wildtype zebrafish. Lines with similar expression pattern will be used for screen system validation studies. *In vitro* promoter studies of the promoter transposon vectors in HepG2 cells have been done to test the regulatory differences between the 3.6kb and the 0.8kb promoter constructs. Insulin, glucagon and metformin treatment will further validate the regulatory differences between the promoter constructs. The development of a screening system that combines both the potential of PEPCK as drug target with the profound advantages of the zebrafish model would have a clear impact in anti-diabetic drug discovery and development.

E7. TUMOUR-NECROSIS FACTOR-α ABROGATES THE MUSCARINIC INDUCED SECRETION OF GLUCAGON-LIKE PEPTIDE-1 FROM THE MGLUTAG L CELL LINE

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Glucagon-like peptide-1 (GLP-1) is an intestinal L cell hormone released in response to nutrient ingestion. GLP-1 acts directly on the pancreatic β-cell, enhancing glucose-stimulated insulin secretion. As well, GLP-1 suppresses glucagon release, gastric emptying and appetite. As a consequence of these biological actions, both long-acting GLP-1 receptor agonists and GLP-1 degradation inhibitors are now used in the clinic for the treatment of patients with type 2 diabetes (T2D). Systemic inflammation is a known consequence of several pathologies, including T2D. Enhanced levels of cytokines, most notably, tumour necrosis alpha (TNFα) are common to both obesity and T2D. Recently, we have reported that nutrient-induced GLP-1 secretion is reduced in rodents under conditions of obesity and insulin resistance; specifically, there is loss of the vagally-mediated, nutrient-induced secretion of GLP-1 in these animals. These findings are consistent with those made in humans with obesity and T2D. We hypothesized that TNFα exerts direct inhibitory effects on the intestinal L cell to induce this response. Consistent with this hypothesis, we and others have demonstrated that the intestinal L cell is a direct target of other adipokines/cytokines, including leptin and interleukin-6. We have now demonstrated that the murine GLUTag L cell line expresses mRNA transcripts for TNFα as well as isoforms 1 and 2 of the TNFα receptor. Pre-treatment of the cells for 24 hr with 50 ng/ml TNFα was not cytotoxic to the cells. However, basal 2-hr GLP-1 release was increased by TNFα pre-treatment (to 122 ± 6% of control; $P<0.01$), whereas GLP-1 secretion induced by treatment with 1 mM of the muscarinic agonist, carbachol (to 131 ± 8%; $P<0.001$), was completely abrogated. These findings suggest a novel means by which systemic inflammation directly influences glucose homeostasis, through impairments in the release of GLP-1.

E8. GLUCOSE SENSING IN mHYPOA-GnRH/GFP IMMORTALIZED HYPOTHALAMIC NEURONS.

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The hypothalamus plays a vital role in maintaining physiological homeostasis through the neuroendocrine axis in response to peripheral nutrient signals. Glucose, the most abundant nutrient in the brain, provides nourishment to neural tissue and signals to specific neurons to maintain energy–balance and reproductive function. To investigate the direct cellular and molecular effects of glucose levels on the reproductive axis, we have generated an immortalized adult-derived neuronal cell line using primary cultures from transgenic mice expressing green fluorescent protein (GFP). These cell lines consist of the entire populations of Gonadotropin-Releasing Hormone (GnRH) neurons within the hypothalamus. Primary hypothalamic cultures were immortalized using simian virus (SV40) large T antigen and a neomycin resistance gene. Following immortalization, cells were FAC-sorted based on GFP fluorescence with greater than 95% purity. As the master regulator of the reproductive axis, GnRH synthesis is required for fertility in both males and females and is tightly regulated by peripheral nutrient signals. Therefore, we hypothesize that an increase in glucose levels will have stimulatory effects on GnRH production, further we hypothesize that prolonged exposure to high glucose levels will dampen this effect, due to the onset of glucose insensitivity. We have screened our cells using RT-PCR for the presence of cellular machinery responsible for sensing of glucose in other tissues, including glucokinase, hexokinase, GLUTs, and the subunits of ATP-sensitive potassium channels (K-ATP; Sur1, Kir6.2), providing evidence that our cell lines utilizes neuronal mechanisms of glucose sensing. We have demonstrated that re-challenging GnRH neurons in media containing 5mM glucose following starvation in 0.5 mM glucose causes excitation of these cells and a significant increase in both c-Fos (15, 30, 60 min) and GnRH mRNA (1 hr, 2hr, 4 hr and 8 hr) levels. Furthermore, we have confirmed that the increase in GnRH mRNA levels following glucose re-challenge is metabolism and concentration dependent, via 2-deoxyglucose studies. Western blot analysis indicates that glucose acts through the second messengers AMP-activated protein kinase (AMPK) and acetyl-coenzyme A carboxylase (ACC), suggesting an AMPK dependant mechanism. Future studies will determine whether high glucose levels induce GnRH secretion, and if this effect can be blunted in a state of glucose insensitivity. Furthermore, we will begin analysis of the transcriptional mechanisms involved in GnRH regulation. Sequential deletions of the GnRH promoter linked to a luciferase reporter gene are already available in our lab, and will be utilized in transient transfection experiments. These studies will provide further insight into how nutrient status may influence neurons involved in reproductive function and will allow delineation of the molecular events involved in this process.

E9. USING MOLECULAR PROFILING TO CHARACTERIZE HUMAN EMBRYONIC STEM CELL-DERIVED HORMONE-POSITIVE AND HORMONE-NEGATIVE CELLS

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It is possible to generate islet hormone expressing cells from hESCs *in vitro*; however, it has been demonstrated that these hormone-expressing cells mature into α cells when transplanted, and that a subpopulation of the non-hormone expressing pancreatic endoderm fraction produce β cells *in vivo* (Kelly et al., 2011). In this study we aim to 1) characterize hormone-

expressing hESC-derived cells to determine why these cells adopt and α cell fate *in vivo*, and 2) characterize the hormone-negative hESC-derived cells to define the composition of this population. hESCs were differentiated *in vitro* from a genetically modified line expressing GFP under the insulin promoter. FACS was used to isolate purified insulin-positive (Ins+) or insulin-negative (Ins-) cells. QPCR established that our Ins- population was in fact hormone-negative. In all cases cells were compared to adult human islets. We performed molecular characterization using microarray and LC/MS/MS to identify differentially expressed genes and proteins. To validate our findings, we performed functional characterization including immunohistochemistry, secretion assays, electron microscopy, and electrophysiology. Our Ins+ cells display many α cell characteristics *in vitro* including glucose-stimulated glucagon secretion and a deficiency of functional L-type calcium channels. Glucagon was among the top five most abundantly expressed proteins, and α cell-specific transcription factors had significantly higher expression in Ins+ cells compared to human islets by microarray. Microarray and proteomic analysis revealed a significant deficiency in β cell-specific transcription factors Pax4 and Nkx6.1. Therefore, the Ins+ population is likely adopting an α cell fate *in vivo* due to high expression of α cell-specific factors, which have committed these cells to an α cell lineage *in vitro*. When examining the Ins- fraction, microarray analysis revealed relatively strong expression of pancreatic endoderm markers including Pax6 and NeuroD. Intriguingly, these cells had extremely high expression of liver- and neuronal-specific genes including the apolipoproteins and alphafetoprotein from the liver and β3 tubulin and neuropilins from neurons. Interestingly, when cultured *in vitro*, the Ins- fraction takes on a neuronal phenotype, suggesting that this is the dominant feature in a heterogeneous population.

E10. THE DIRECT EFFECTS OF PALMITATE AND OMEGA-3 FATTY ACIDS ON THE MOLECULAR CIRCADIAN LOCK IN IMMORTALIZED, CLONAL HYPOTHALAMIC NEURONS

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With obesity, excessive nutrient levels can lead to metabolic disturbances and the progression of pathologies, such as diabetes and heart disease. The obese state has also been shown to have a reciprocal relationship with circadian rhythms. Recent studies have demonstrated that atypical circadian rhythms result in obese phenotypes and similarly, metabolic dysregulation negatively shifts the circadian clock. To date, the molecular mechanisms underlying these phenomena are not well understood and are difficult to study in vivo. In order to address this issue, our laboratory has generated an array of immortalized, clonal, neuronal, hypothalamic cell lines through retroviral transfection. Using real time RT-PCR, we have identified that the mHypoE 37 cell line expresses a number of neuropeptides linked to obesity such as neuropeptide Y (NPY), agouti-related peptide (AgRP), urocortin2, ghrelin, as well as important receptors, GPR210, insulin receptor, leptin long-form receptor, hypocretin receptor 1 and 2. It also expresses key circadian molecular components, Bmal1, Per2, and Rev-erbα, all of which oscillate with an approximate 24 hour period. Previously, we have demonstrated that the saturated fatty acid, palmitate (200 μM) attenuates insulin signalling, generates ER stress, induces apoptosis, and increases the orexigenic neuropeptide, NPY. Further, palmitate disrupts cellular circadian gene expression. Conversely, unsaturated omega-3 fatty acids have been shown to have protective effects against the development of insulin resistance. Preliminary studies indicate that administration of 12 μM of omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), can significantly suppress transcript levels of the orexigenic neuropeptide AgRP. Provided the opposing effects induced by saturated versus unsaturated fatty acids, we hypothosize that palmitate will hinder the circadian molecular clock in the mHypoE 37 cell model, whereas co-treatment with omega-3 fatty acids will have restorative effects. Furthermore, due to the variance in neuronal sensing of saturated and unsaturated fatty acids, we are delineating the differential signalling cascades by which each type of fatty acid alters circadian transcript levels. Our novel hypothalamic cell models serve as optimal models to study the effects of nutrient excess on the cellular circadian clock, thereby further defining the complex interplay between circadian rhythms and metabolic status.

E11. P38 MAPK (MITOGEN ACTIVATED PROTEIN KINASE) MEDIATES LIPID-INDUCED HEPATIC INSULIN RESISTANCE BUT IMPROVES PERIPHERAL INSULIN SENSITIVITY

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Insulin resistance is common in obese individuals and it is also the key factor in the pathogenesis of type 2 diabetes. Expanded adipose tissue from obese individuals increases plasma levels of FFA (free fatty acids) and alters the secretion pattern of adipokines. Both factors contribute to the development of insulin resistance in classical target tissues such as the liver and skeletal muscles. However the exact molecular mechanisms behind the development of FFA-induced hepatic insulin resistance are not completely understood. We have previously shown that p38 MAPK was activated in our short-term lipid infusion model of 7 hours but its inhibition did not prevent lipid-induced hepatic insulin resistance. Therefore, we wondered whether p38 MAPK may be playing a causal role after more prolonged lipid exposure as previously shown in isolated hepatocytes. To address this question, we randomly assigned female Wistar rats to four different infusion groups: saline, IH (Intralipid and heparin to increase FFA), IH and SB239063 (p38 MAPK inhibitor), or SB239063 alone. The right jugular vein and left carotid artery were cannulated for infusion and blood sampling respectively. Insulin sensitivity was assessed near the end of 48-hour infusion, using the hyperinsulinemic-euglycemic clamp. Concomitant glucose tracer was given to separately evaluate hepatic from peripheral insulin sensitivity. We found that co-infusion of SB239063 with IH prevented lipid-induced hepatic insulin resistance ($p<0.05$ IH + SB239063 vs. IH), but aggravated lipid-induced peripheral insulin resistance ($p<0.05$ IH + SB239063 vs. IH). SB239063 infusion alone worsened peripheral insulin sensitivity ($p<0.05$) but did not improve hepatic insulin sensitivity compared to saline. Our results suggest that p38 MAPK activation mediates lipid-induced hepatic insulin resistance but protects against peripheral insulin resistance. This is in agreement with the view that mechanisms of FFA-induced insulin resistance are time and tissue-dependent and that p38 MAPK may be involved in peripheral insulin action.

E12. ROLE OF THE SNARE PROTEINS IN GLUCAGON-LIKE PEPTIDE-1 SECRETION

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Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted by enteroendocrine L-cells of the distal gut in response to vagal and endocrine stimulation, as well as direct contact with nutrients. GLP-1 activates its receptor on the β-cell and stimulates glucose-dependent insulin secretion. Little is known about mechanisms regulating GLP-1 secretion by these cells. GLP-1 is stored in secretory granules, which must fuse with the cell membrane for exocytosis to occur. This is likely mediated through a SNARE-dependent pathway, though this has not been examined in the L-cell. The SNARE complex consists of three proteins: SNAP and Syntaxin, located on the plasma membrane; and VAMP, located on the granule. RT-PCR and immunoblot indicated that the SNARE isoforms, SNAP25, Syntaxin1, and VAMP2 are expressed in GLUTag cells, a well characterized murine cell model of the L-cell. Thus, I hypothesize that SNAP25, Syntaxin1, and VAMP2 are essential components in the exocytosis of GLP-1. Botulinum and Tetanus are neurotoxins that potently cleave SNARE proteins (Botulinum A cleaves SNAP25, Botulinum C1 cleaves SNAP 25 and syntaxin1, and Tetanus cleaves VAMP2). GLUTag cells were transfected with plasmids containing constructs for the neurotoxins, and reached ~90% transfection efficiency. Neurotoxin cleavage of the appropriate SNARE complex proteins will be confirmed by immunoblot. Transfected GLUTag cells will be treated with known GLP-1 secretagogues, and the importance of SNARE complex proteins in the L-cell exocytotic pathway will be evaluated via radioimmunoassay. Secretion of GLP-1 can be induced via several different pathways. Secretagogues chosen to evaluate for GLP-1 secretion include: GIP, for PKA; bethanechol, for classical and novel PKCs; oleate, for PKCζ), and have been found to greatly induce secretion of GLP-1. Elucidating molecular mechanisms involved in GLP-1 secretion will enhance understanding of L-cell function, and aid in the development of novel targets for the treatment for T2D.

E13. EFFECT OF INSULIN ANALOGUES ON CANCER PROMOTION

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Solid epidemiological studies support the link between insulin treatment of diabetes patients and increased cancer risk. While more favorable insulin analogues are 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 colon cancer to investigate the cancer promoting effect of insulin glargine, which is the analogue that has been mostly associated with cancer. 3 wks after the MNU injection (50mg/kg) and 2 wks after the first AOM injection (15mg/kg; twice), the Sprague-Dawley female rats were assigned randomly to 3 groups that were treated with vehicle (saline), NPH (unmodified insulin) or glargine. The blood glucose level decreased to the same extent with NPH and glargine (by about 3mmol/L) 4-5hr after the insulin injection (peak insulin action). All the rats were sacrificed after 6 wks of insulin/vehicle injections. The rats treated with NPH insulin (n=6) tended to have a higher mammary tumour incidence and multiplicity (number of tumours per animal) and had a significantly higher tumour volume than those on vehicle treatment (n=6). Unexpectedly, glargine-treated rats (n=6) had mammary tumour incidence, multiplicity and volume comparable to the vehicle-treated rats. There was no effect of NPH on total aberrant crypt foci (ACF: precursor of colon cancer) number**.** However, there was a significant effect of NPH on ACF multiplicity (number of crypts per ACF, $P<0.05$)**.** Glargine-treated rats did not differ from vehicle treated rats in either ACF number or multiplicity. In this study, we did not observe any increase in promotion of either mammary cancer or ACF number by insulin glargine. Nonetheless, these results must be confirmed with an increased n.

E14. UNCOUPLING PROTEIN 2 (UCP2) REGULATES INSULIN SECRETION AND GLUCOSE HOMEOSTASIS

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The function of uncoupling protein 2 (UCP2), particularly in the control of β cell function and glucose homeostasis, has been debated over the last decade. Although many studies suggest that UCP2 expression is negatively associated with insulin secretion, the precise mechanism is still unclear. Some argues that UCP2 controls insulin secretion via regulating ATP production, while others contend that UCP2 modulates of reactive oxygen species (ROS) signals responsible for the amplification of insulin secretory pathways. Much of our understanding of β cell UCP2 function comes from two types of mouse models, whole-body UCP2 knockout model (UCP2-/-) and β-cell specific UCP2 knockout (UCP2 BKO) model. However, there are fundamental issues with each of these models when studying UCP2 function specifically in the β cell. The UCP2-/- model is problematic because of the wide distribution of UCP2 throughout the body. To circumvent this, we generated a β cell-specific UCP2 deletion model (UCP2 BKO) using the cre-lox system driven by the rat-insulin promoter (RIP). However, in this model, UCP2 deletion occurs at embryonic stage and ectopic RIPCre expression has been demonstrated in hypothalamus. Therefore, we have generated a novel β cell-specific inducible UCP2 knock out mouse model (MIPCreER×loxUCP2), which has a less chronic and more β-cell-specific UCP2 deletion, to study UCP2 function specifically in the β cell. MIPCreER×loxUCP2 mice are generated by crossing loxUCP2 mice with mice that express Cre recombinase-estrogen receptor (ER) fusion protein driven by mouse insulin promoter (MIP). MIPCreER×loxUCP2 mice were either intraperitoneally injected with tamoxifen to induce β cell specific UCP2 deletion (inducible BKO, iBKO) or with corn oil placebo (CO). Various *in vitro* and *in vivo* parameters were measured, including islet cell mitochondrial membrane potential, whole islet ROS and ATP levels, glucose stimulated insulin secretion (GSIS), glucose and insulin tolerance, and plasma hormone levels. iBKO islets display unchanged islet ATP content and lower ROS levels but tend to secrete more insulin upon glucose stimulation ($p=0.0801$). iBKO and CO mice display similar levels of glucose and insulin tolerance. Interestingly, however, iBKO mice sustained significantly higher plasma insulin levels during glucose tolerance test ($p<0.05$). These results indicate that our iBKO mice display different phenotype from the UCP2 BKO mice. By using this novel inducible β-cell-specific UCP2 knockout model, we have shed light

on the function of UCP2 on primary β cells and our study may further support the notion that UCP2 does not behave as a classic uncoupling protein in the regulation of insulin secretion.

E15. THE ROLES OF IKKβ AND 'β-CELL INSULIN RESISTANCE' IN β-CELL LIPOTOXICITY *IN VIVO*

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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 a known activator of IκBα Kinase β (IKKβ), which, by phosphorylating the inhibitor IκBα, activates the proinflammatory transcription factor nuclear factor κB (NFκB). IKKβ is also known to phosphorylate serine/threonine residues of insulin receptor substrates (IRS), which impairs insulin-like growth factor-1 (IGF-1)/insulin signaling. Data in our lab obtained with the IKKβ inhibitor salicylate suggest that IKKβ activation is causally involved in β-cell lipotoxicity, as salicylate restored β-cell function. Preliminary data using bisperoxovanadate (tyrosine phosphatase inhibitor), which prevented β-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 IKKβ-mediated 'β-cell insulin resistance' plays a causal role in fat-induced β cell dysfunction we used mice with β-cell specific knockout of either IKKβ or PTEN (negative regulator of insulin signaling). Knockout mice and littermate controls were intravenously infused with saline or oleate for 48h, followed by assessment of β-cell function in vivo using a hyperglycaemic clamp (22mM). 48h fat infusion impaired β-cell function (assessed by the Disposition Index: DI) compared to saline infusion in control mice. In contrast, PTEN-null mice were protected from the oleate-mediated decrease in DI. Oleate infusion decreased DI in IKKβ-null mice, however insulin clearance decreased. This resulted in higher plasma insulin levels and thus the decreased DI may be due to β-cell rest rather than dysfunction. These preliminary data suggest that 'β-cell insulin resistance' may contribute to FFA-mediated β-cell dysfunction, however the role of IKKβ is unclear.

E16. GENERATION OF IMMORTAL HUMAN ISLETS VIA A VIRAL AND GROWTH FACTOR APPROACH

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The increasing prevalence of diabetes mellitus dictates the necessity of a relevant islet cell model for both basic research and therapy. However, there are currently no human cell lines available for in vitro research and the availability of primary islets is scarce. Fundamental differences in islet cyto-architecture and ion channel function make rodent islet cell lines not optimal for diabetes research. Thus, we propose a viral and growth factor approach to generate immortalized islet cells. Healthy human donor islets were isolated and infected with a viral construct containing the Large T-Antigen pre- or post-dispersion. Islet cells were treated with specific growth factors, such as hepatocyte growth factor and Exendin-4, either prior to or after viral infection. Initial immunocytochemistry (ICC) imaging of primary islet cultures was able to identify alpha, beta, delta and pancreatic polypeptide subtypes of primary islet cells; proliferation marker Ki67 staining was negative for all. However, cells of neuronal origin stained with neurofilament also stained positive for Ki67, and were observed to proliferate in culture. Retroviral infected islet cells that survived geneticin selection were observed to proliferate slowly. The immortalized cells were observed to detach from the culture surface and form loose aggregates of cells. ICC imaging could not be utilized due to the inability of the infected cells to remain attached to Poly-L-Lysine coated surfaces. Subsequent experiments with Matrigel matrix indicated that the immortalized islets were able to attach to the matrix at a slow rate. Furthermore, insulin radioimmunoassay was able to detect insulin, and therefore indicates that the immortalized islet cells secrete basal insulin and are potentially glucose responsive. Analysis of glucose stimulated insulin secretion is underway. Additional experiments are in progress to optimize the immortalization process and improve proliferation rate. Given that the lack of human islet cell lines has slowed progress in human diabetes research, success in this approach may significantly increase opportunities for basic islet cell research and eventually therapeutic avenues.

E17. ACUTE DELETION OF SIRT1 IN PANCREATIC BETA CELLS CAUSES IMPAIRED GLUCOSE TOLERANCE AND DECREASED INSULIN SECRETION IN MICE

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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 SirT1 dysfunction 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. 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. To gain mechanistic insight, SirT1 knockdown in Min6 cells resulted in increased expression of mitochondrial genes, Pgc1α, Cox2, and Pparγ, thereby altering mitochondrial dynamics ultimately affecting insulin secretion. These data highlight SirT1's role in insulin secretion and its potential for therapeutic use in diabetic patients.



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