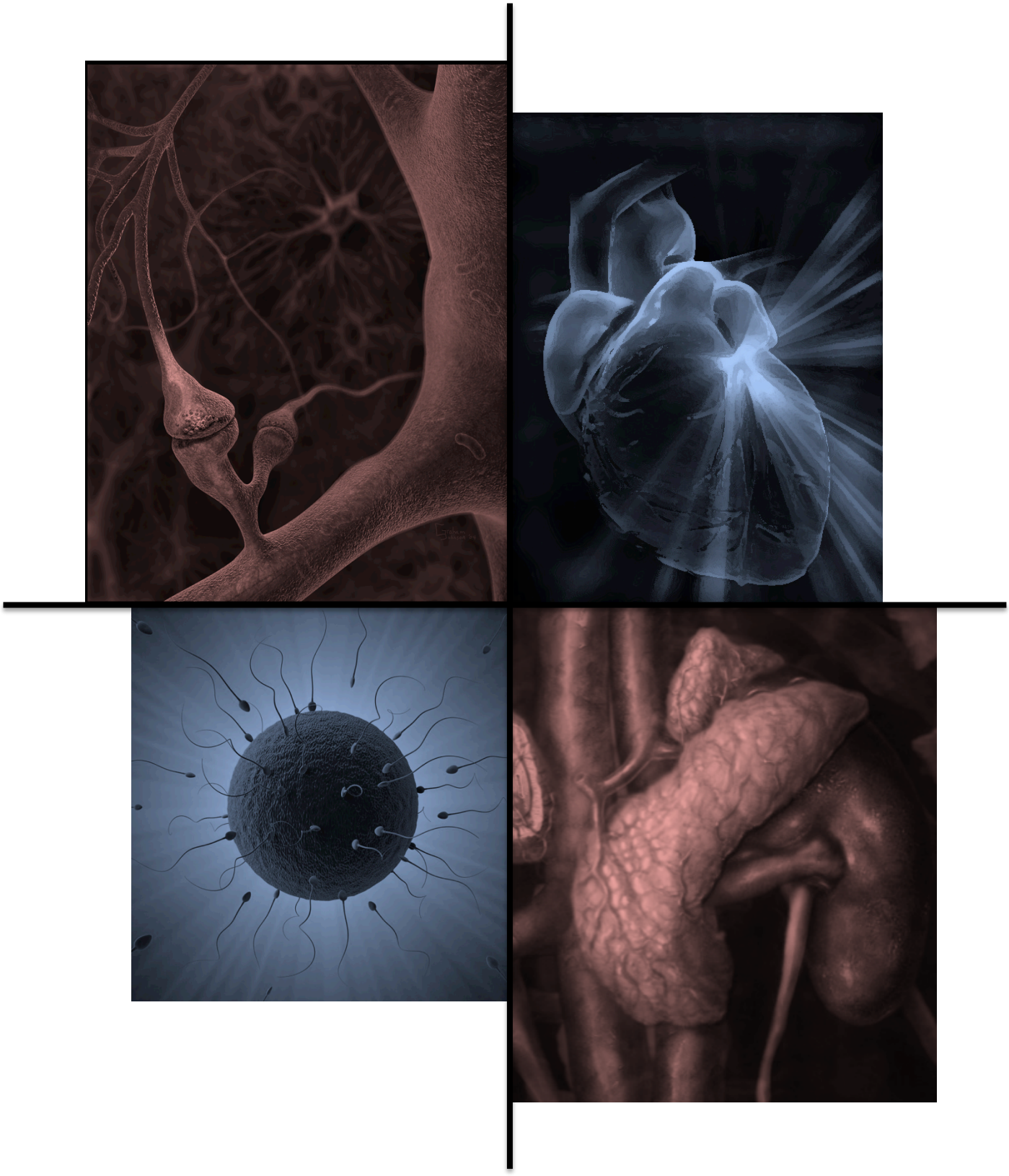
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KEYNOTE SPEAKER

Dr. Bruce M. Spiegelman

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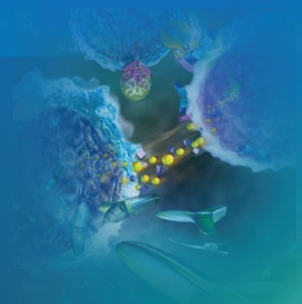
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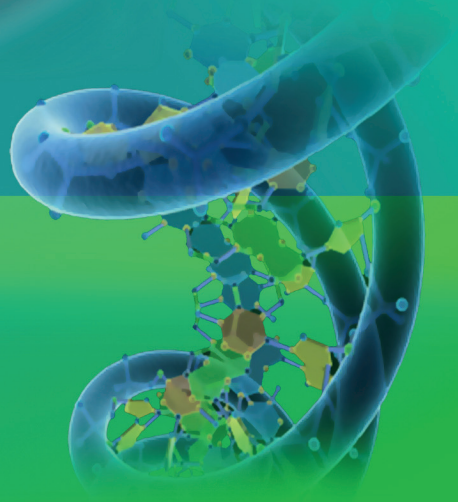
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31th Annual Frontiers in Physiology Symposium

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

8:50-9:00 **Opening Remarks**- Tom Lu and Dr. Stephen Matthews (MSB 2172)

9:00-9:45 **Endocrine and Diabetes Platform Oral Presentations** (MSB 2172)
MC: Krishana Sankar

Lauren Hager	Lecithin: cholesterol acyltransferase (LCAT) deficient mice are resistant to hepatic endoplasmic reticulum (ER) stress and insulin resistance through evasion of cholesterol accumulation in the ER.
Katie Rowland	Intestinal epithelial insulin-like growth factor-1 receptor signalling is essential for chronic glucagon-like peptide-2-induced epithelial proliferation and associated growth of the crypt-villus unit.
Akansha Tiwari	Elucidating the role of stromal cell derived factor 2 like-1 (SDF2L1) in the endoplasmic reticulum (ER) stress response in pancreatic β-cells.

9:45-10:00 **Break**

10:00-10:45 **Reproduction and Development Platform Oral Presentations** (MSB 2172)
MC: Melanie Audette

Jeff Emack	Chronic maternal adversity modifies activity and attention behaviours in juvenile guinea pig offspring: dopaminergic modulation.
Jinny Kim	Suppressor of fused controls murine cerebellar morphogenesis via GLI3 repressor.
Han Li	Role of placental VEGFA in maternal function during pregnancy

10:45-11:00 **Break**

11:00 -12:00 **KEYNOTE LECTURE** (MacLeod Auditorium)
"Adipogenesis, PPAR*gamma* and Therapeutics: A New Look at an Old Friend"
Bruce M. Spiegelman, Ph.D
Stanley J. Korsmeyer Professor of Cell Biology and Medicine
Dana-Farber Cancer Institute, Harvard Medical School

31th Annual Frontiers in Physiology Symposium (Cont.)

12:00-1:30 **Poster Viewing and Lunch** (Stone Lobby)

1:30 **Departmental Photograph** (Outside MSB stairs)

1:30-2:00 **Poster Viewing** (Stone Lobby)

2:00-2:45 **Cardiovascular Platform Oral Presentation** (MSB 2172)
MC: Richard Gao

Roxy Chis	Elucidation of the protective mechanism of α crystalline B in cardiomyocytes.
Jeff Kroetsch	TNFα is central to the augmented myogenic response of skeletal muscle resistance arteries in heart failure.
Mariel Van Woudenberg	Blood pressure reactivity to physical and mental stress in adolescence: the role of sex, puberty stage and visceral and whole-body adiposity.

2:45-3:00 **Break**

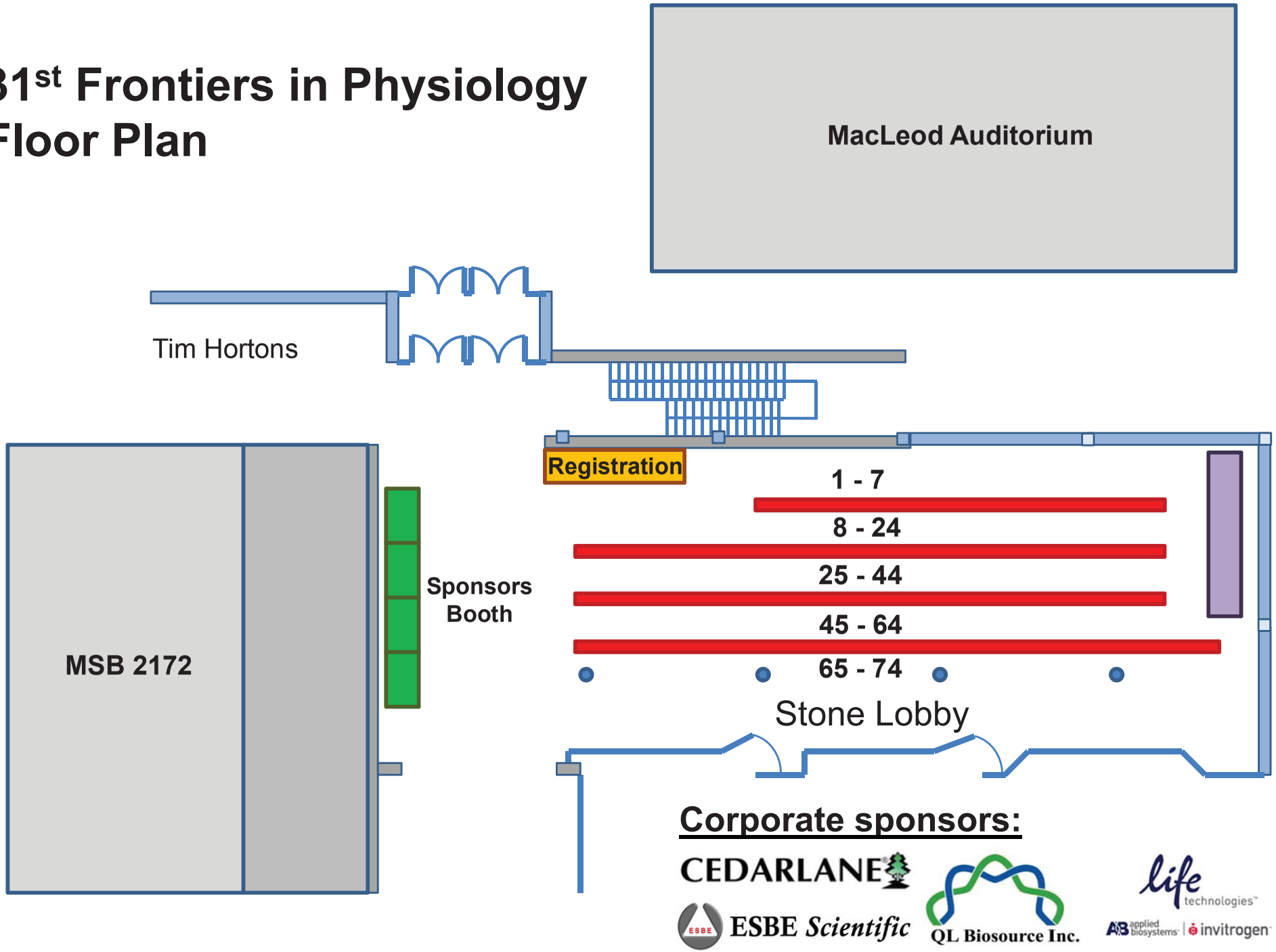
3:00-3:45 **B.R.A.I.N. Platform Oral Presentations** (MSB 2172)
MC: William To

Lulu Gao	Synergistic effects of stroke and alzheimer's pathologies on neuronal survival of adult born neurons in the hippocampus.
Nathalie Goodfellow	A novel 5-HT receptor subtype in rodent prefrontal cortex: the 5-HT5a receptor.
Paul Whissell	The role of δGABAARs in cognition and synaptic plasticity.

3:45-4:00 **Break**

4:00-6:00 **Awards Ceremony and Reception** (Outside of MacLeod Auditorium)

31st Frontiers in Physiology Floor Plan



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Keynote Lecture



Adipogenesis, PPAR*gamma* and Therapeutics:

A New Look at an Old Friend

Bruce M. Spiegelman, Ph.D

Bruce M. Spiegelman is the Stanley J. Korsmeyer Professor of Cell Biology and Medicine at Harvard Medical School and Dana-Farber Cancer Institute. Dr.Spiegelman received a B.S. with highest honors from the College of William and Mary in 1974, his PhD in biochemistry from Princeton University in 1978, and completed postdoctoral work at MIT. He joined Harvard Medical School and Dana-Farber Cancer Institute in 1982. His research focuses on fat cell biology, diabetes and muscular diseases. Dr.Spiegelman has been honored with many awards including Bristol-Myers Squibb Award for Distinguished Achievement in Metabolic Research; the Solomon Berson Award, American Physiological Society; the Rolf Luft Award in Endocrinology, Karolinska Institute (Sweden); the Trans-Atlantic Medal, British Endocrine Society; the Naomi Berrie Award for Outstanding Achievement in Diabetes Research, Columbia University. In 2002 Dr. Spiegelman was elected to the American Academy of Arts and Sciences and the National Academy of Science.

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MESSAGE FROM THE CHAIR OF FRONTIERS IN PHYSIOLOGY (FIP)

On behave of the Graduate Association of Students in Physiology (GASP), it is my great pleasure to welcome everyone to the 31st 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.

Every year, the Department of Physiology welcomes a distinguished scientist to give a keynote lecture at FIP. This year we are honoured to welcome **Dr. Bruce M. Spiegelman**. Dr. Spiegelman is a professor of Cell Biology at the Harvard Medical School. His research focuses on the gene transcription mechanisms in regulating energy homeostasis. His work has potential application in developing novel therapeutic strategies for diabetes, obesity, muscular and neurodegenerative diseases. We are delighted to have Dr. Spiegelman deliver his keynote lecture this morning, titled "*Adipogenesis*, PPAR*gamma and Therapeutics: A New Look at an Old Friend*".

The events of today were made largely possible by the combined efforts of a fantastic team. First and foremost, I would like to thank all the GASP members for their dedication toward a successful symposium. I would also like to thank the Chair of the department, Dr. Stephen Matthews, and to the graduate coordinators, Drs. Denise Belsham and Martin Wojtowicz, for their continuing support of our student association. Many thanks are extended to the administrative staffs of the Department of Physiology who also helped to ensure a successful FIP day. A special thanks to 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: all four Department of Physiology research platforms, University of Toronto Faculty of Medicine, University of Toronto Neuroscience Program, Division of Teaching Labs, Graduate Students' Union, Invitrogen, Cedarlane Laboratories, QL Biosources Inc, and ESBE.

We are delighted to welcome you to FIP.

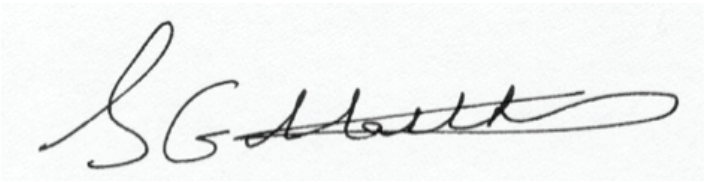
Tom Z. Lu
Vice-President of Graduate Association of Students in Physiology
Frontiers in Physiology Chair

MESSAGE FROM CHAIR OF THE DEPARTMENT OF PHYSIOLOGY

On behalf of the Department of Physiology, it is a very great pleasure to welcome you the annual **"Frontiers in Physiology Research Day"**. This Symposium speaks highly of our graduate students who have organized this event for the last 31 years. The strength of our Department derives from the enthusiasm, originality and creativity of the individuals whose collective efforts are responsible for its viability. All of these attributes are ably represented in the youngest and most stimulating individuals in the Department – our graduate students and post-doctoral fellows. This is the day when they have the opportunity to demonstrate their considerable and varied scientific prowess and many accomplishments.

Special thanks and recognition is due today to **Mr. Tom Lu**, FIP Chair and Vice-President of the Graduate Association of Students in Physiology (GASP). Tom and his team have done an outstanding job and have generated an excellent program. Please join me in also thanking **Mr. Keith Ho**, President of GASP.

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



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

Oral Presentations

Endocrinology and Diabetes Platform

ELUCIDATING THE ROLE OF STROMAL CELL DERIVED FACTOR 2 LIKE-1 (SDF2L1) IN THE ENDOPLASMIC RETICULUM (ER) STRESS RESPONSE IN PANCREATIC B-CELLS.

A. Tiwari1, A. Volchuk1,2,3. Departments of Physiology1 and Biochemistry2, Faculty of Medicine3, University of Toronto.

Type 2 diabetes is characterized by insulin resistance and pancreatic β-cell failure. Insulin resistance leads to increased insulin demand and increased protein misfolding in the ER. The accumulation of the misfolded proteins in the ER can cause ER stress, which can lead to pancreatic β-cell dysfunction. Cells respond to ER stress by the unfolded protein response (UPR), which increases protein folding capacity and causes degradation of misfolded proteins through the ERAD pathway. To study the ER stress response in pancreatic β-cells we used an insulinoma cell line with inducible expression of a mutant proinsulin. Expression of proinsulin-C96Y tagged with GFP caused ER stress and the induction of numerous unfolded protein response genes. One of the most highly induced genes was Stromal Cell-Derived Factor 2 Like 1 (SDF2L1), an ER stress-inducible soluble protein localized to the ER, with an as yet unknown function. To elucidate the role of SDF2L1 in the ER stress response in pancreatic β-cells we have undertaken several experimental approaches to identify SDF2L1 interacting proteins and examine if it is required for cell survival under ER stress conditions. Immunoprecipitation studies have shown a direct association of SDF2L1 with the ER chaperone proteins GRP78. We also found that SDF2L1 can interact with misfolded insulin, although the association is indirect. These results suggest that SDF2L1 may act as a co-chaperone protein to either mediate protein folding or potentially aid in the degradation of terminally misfolded proteins. siRNA-mediated knock-down of SDF2L1 however, did not affect steady-state mutant insulin levels, likely precluding a role in misfolded protein degradation. However, SDF2L1 appears to be essential in the ER stress response as knockdown of SDF2L1 significantly increased ER stress-induced cell apoptosis as compared to control siRNA. Studies are currently underway to identify additional SDF2L1 interacting proteins to identify the function of SDF2L1 in the ER stress response in pancreatic β-cells.
(This study was funded by the Canadian Institute for Health Research and Banting and Best Diabetes Centre).

INTESTINAL EPITHELIAL INSULIN-LIKE GROWTH FACTOR-1 RECEPTOR SIGNALING IS ESSENTIAL FOR CHRONIC GLUCAGON-LIKE PEPTIDE-2-INDUCED EPITHELIAL PROLIFERATION AND ASSOCIATED GROWTH OF THE CRYPT-VILLUS UNIT.

K.J. Rowland1, D. Lee1, K. Wan1, P.L. Brubaker1,2. Departments of Physiology1and Medicine2, Faculty of Medicine, University of Toronto, Toronto.

TGlucagon-like peptide-2 (GLP-2) is an intestinal-specific mitogenic peptide. In vivo studies have previously demonstrated that GLP-2 requires insulin-like growth factor-1 (IGF-1) to induce its intestinotrophic effects. Moreover, we have confirmed that expression of the IGF-1 receptor (R) specifically in intestinal epithelial cells (IEC) is required for the acute proliferative effects of GLP-2. Therefore, we hypothesized that small intestinal growth responses to chronic GLP-2 also occur through an IEC IGF-1R-dependent pathway. Villin-Cre-ERT2+/0 and Igf1rflox/flox mice were bred to achieve homozygous deletion of IGF-1R in the IECs upon induction with tamoxifen. IE-igf1rKO and control (Igf1rflox/flox and villin-Cre-ERT2+/0) mice were treated with GLP-2 or saline for 10 d. RESULTS: IE-igf1rKO mice displayed decreased mRNA expression levels of IGF-1R ($p<0.05$) and proglucagon ($p<0.05$) in jejunal mucosa relative to controls. However, no significant differences were observed in mRNA levels of IGF-1, GLP-2R, the ErbB ligands epiregulin, amphiregulin and HB-EGF, and ErbB receptors in jejunal mucosa of control and IE-igf1rKO mice 10 d after tamoxifen treatment. Chronic administration of GLP-2 to villin-Cre-ERT2+/0 control mice significantly increased small intestinal weight ($p<0.01$), crypt-villus height ($p<0.01$), and crypt cell proliferation ($p<0.05$) as compared to saline controls, in both tamoxifen- and vehicle-pretreated mice, indicating no effects of either Cre-recombinase or tamoxifen on GLP-2-induced gut growth. No significant differences were detected between the body weights and mucosal cross-sectional area of vehicle- or GLP-2-treated control and IE-igf1rKO mice. Control and knockout mice also demonstrated an increase in small intestinal weight with GLP-2 treatment ($p<0.05$ for control and $p<0.01$ for IE-igf1rKO animals), as compared to vehicle-treated animals. However, IE-igf1rKO mice exhibited a marked decrease in GLP-2-induced crypt-villus height ($p<0.05$) and in the number of Ki67+-proliferating cells ($p<0.05$)

following GLP-2 treatment, relative to controls. These findings demonstrate that selective expression of IGF-1R in IECs is essential for the proliferative effects of chronic GLP-2 administration.
(This study is funded by the Canadian Institute for Health Research - PLB: MOP- 9940. KJR is supported by a CIHR/ Canadian Digestive Health Foundation joint doctoral award, and PLB by the Canada Research Chair Program.)

LECITHIN:CHOLESTEROL ACYLTRANSFERASE (LCAT) DEFICIENT MICE ARE RESISTANT TO HEPATIC ENDOPLASMIC RETICULUM (ER) STRESS AND INSULIN RESISTANCE THROUGH EVASION OF CHOLESTEROL ACCUMULATION IN THE ER

L. Hager1,2, L. Li2, L. Liu2, G. Maguire2, M. Naples3, C. Baker3, L. Magomedova4, C.L. Cummins4, P.W. Connelly2 (ADA), K. Adeli3 (ADA), D.S. Ng1,2. Department of Physiology, Faculty of Medicine, University of Toronto, Toronto1, Keenan Research Centre, Li Ka Shing Knowledge Institute, Department of Medicine, St Michael's Hospital, Toronto2, Division of Clinical Biochemistry, Hospital for Sick Children, Toronto3, Faculty of Pharmacy, University of Toronto, Toronto4.

R-spondin1 (Rspo1) is an intestinal growth factor that acts through the canonical Wnt (cWnt) signaling pathway leading to induction of cWnt target genes (i.e. c-myc). We have previously demonstrated Rspo1 expression in murine islets and β-cell lines by qRT-PCR and/or immunoblot, and established that Rspo1 stimulates proliferation and insulin secretion, and inhibits cytokine-induced apoptosis in MIN6 cells. We thus investigated the role of Rspo1 in -cells in vivo using Rspo1 knockout (Rspo1KO) mice. Rspo1KO mice had normal fasting glycemia and demonstrated no differences in body and pancreatic weights compared to wild-type (WT) mice. Rspo1KO mice had better glucose tolerance after an oral glucose challenge compared to WT mice with 1.2-fold reduction in area-under-the-curve over 120 min (AUC120min), but an insulin tolerance test showed no difference in their insulin sensitivity. In contrast to our previously reported proliferative effects of Rspo1 in vitro, Rspo1 deficiency in mice resulted in a 2.7-fold increase in β-cell mass and this was associated with 1.5-fold increase in the number of intermediate-sized islets, and a 3-fold increase in Ki67-positive -cells, a marker of proliferation, but no change in -cell apoptosis in Rspo1KO relative to WT mice. Unexpectedly, Rspo1KO pancreas demonstrated the presence of insulin-positive ductal cells which were absent in the WT pancreas. Since we have previously established an interaction between Exendin-4 (EX4), a glucagon-like peptide-1 receptor agonist, and Rspo1 in vitro, we determined in vivo the effect of 2-week EX4 treatment in Rspo1KO mice, showing a 1.2-fold reduction in AUC120min relative to EX4-treated WT mice after an oral glucose challenge. EX4-treated Rspo1KO mice were also more insulin sensitive compared to EX4-treated WT mice. Interestingly, administration of EX4 to WT and Rspo1KO mice caused no differences in β-cell mass. The present findings therefore reveal a novel role for Rspo1 as a regulator of β-cell behaviour in vivo.

Reproduction and Development

ROLE OF PLACENTAL VEGFA IN MATERNAL FUNCTION DURING PREGANCY.

H. Li1,2,3, D. Qu1, H. Sung1, A. Nagy1,4 , S Lee Adamson1,2,3. Samuel Lunenfeld Research Institute1, Mount Sinai Hospital; Department of Physiology2, University of Toronto; Obstetrics and Gynecology3, University of Toronto and Molecular Genetics4, University of Toronto, Toronto.

Introduction: Maternal circulating VEGFA increases during pregnancy however its source and function are largely unknown. VEGFA is expressed in placenta. In mice, Vegfa mRNA is highest in the placental junctional zone (JZ) which is perfused by blood returning to the maternal circulation. We hypothesized that the JZ is a source of maternal plasma VEGFA and knocking out one allele of Vegfa in the JZ (JZ-Vegfa+/-) of every placenta would affect maternal function during pregnancy. Methods: To obtain dams carrying JZ-Vegfa+/-placentas, we bred homozygous Vegf-loxP females with homozygous Tpbpa-Cre males (Tpbp is JZ-specific). For controls, we bred Vegf-loxP females with wild type males. At 17.5d (i.e. near term), we measured maternal circulating VEGFA by ELISA (n=9 per group). We also measured maternal arterial pressure using a catheter (n=6), maternal cardiac output (CO) with ultrasound, protein, red blood cells and glucose in maternal urine using test strips, and assessed litter size, and fetal body weight (all n=11). Results: Contrary to our hypothesis, VEGFA in maternal circulation was 25% higher in dams carrying JZ-Vegfa+/-placentas (560±20pg/ml vs. 450±30pg/ml, $p < 0.05$). Interestingly, maternal arterial pressure was significantly decreased in these mice (62±3mmHg vs. 78±1mmHg, $p < 0.05$) while CO was unaltered suggesting lower peripheral vascular resistance. Hematuria was found in 6 of 11 experimental mice and in 0 of 9 controls ($p < 0.05$) with no other abnormalities in maternal urine. Nevertheless, litter size and fetal weights did not differ between groups. Discussion:The paradoxical increase in VEGFA in maternal plasma may cause vasodilation and thereby play a role in the decrease in peripheral vascular resistance in dams carrying JZ-Vegfa+/-placentas. Although the mechanisms remain to be defined, results suggest that placental-JZ VEGFA is important in maternal function during pregnancy. Supported by CIHR MOP-93618.

CHRONIC MATERNAL ADVERSITY MODIFIES ACTIVITY AND ATTENTION BEHAVIOURS IN JUVENILE GUINEA PIG OFFSPRING: DOPAMINERGIC MODULATION.

J. Emack1, S. Matthews1,2,3. Departments of Physiology1, Obstetrics and Gynecology2, and Medicine3, Faculty of Medicine, University of Toronto.

Objective: Maternal adversity during the perinatal period has been linked to attentional and behavioral problems in children, as well as increased adrenocortical activity. Previously, we demonstrated juvenile offspring of mothers exposed to chronic maternal adversity (CMA) display elevated basal adrenocortical activity. CMA also increased locomotor activity in adult male offspring and decreased attention in adult female offspring. Given that these alterations in behavior typically emerge in children and are linked to modified central dopamine signaling, we hypothesized that; 1) CMA increases locomotor activity and decreases attention in juvenile male and female offspring and, 2) administration of d-amphetamine (AMPH) will reverse these behavioral effects. Methods: Guinea pigs were exposed to a sequence of mild/moderate stressors every other day over the second half of gestation until weaning on postnatal day 25 (n=8). Control animals remained undisturbed (n=8). After weaning, locomotor activity was assessed in novel (open-field; 30min) and familiar (home-cage; 24hr) environments in male and female offspring. Attention was assessed using prepulse inhibition (PPI). A subset of animals (n=6-8/gp) was treated with AMPH (1ml/kg, sc) prior to testing. Results: In male offspring, CMA resulted in a significant 1-h phase-shift in the profile of diurnal activity in the home cage environment. In contrast, CMA decreased activity in the novel open-field ($p<0.05$). There was no effect of CMA on activity in females. Further, CMA did not alter PPI responses in either sex. AMPH increased activity ($p<0.01$) in the home cage, but profoundly reduced activity in the open-field, in both sexes ($p<0.01$). Similar behavioral responses were observed in CMA and control offspring. Interestingly, AMPH treatment increased attention in female offspring, but only in the CMA group ($p<0.01$); a similar trend was observed in males. Conclusion: CMA profoundly alters the diurnal profile of locomotor activity, as well as decreasing exploratory activity in the open field, but this only occurs in males. Interestingly, the effects of AMPH were highly context-dependent, with AMPH causing reduced activity in a novel setting and increased activity in a familiar setting in both sexes. Further, there was no significant interaction of CMA with this response. In the present study, CMA did not significantly modify attentional systems (as determined by PPI), however there was a significant interaction of CMA with the effects of AMPH on attention in female offspring. Clearly, CMA results in complex changes in activity and attention behaviors, aspects of which appear to be modulated by altered dopamine signaling.

SUPPRESSOR OF FUSED CONTROLS MURINE CEREBELLAR MORPHOGENESIS VIA GLI3 REPRESSOR.

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Suppressor of fused (SuFu) is a gene encoding an intracellular protein that regulates the balance between GLI activators and repressors, transcriptional effectors of the Sonic Hedgehog (SHH) signaling pathway. Importantly, SUFU is required for stabilizing 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 uncontrolled proliferation 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. Levels of GLI1 and GLI2 activators were markedly reduced compared with control cerebellar lysates, and GLI3R protein was barely detectable in the absence of SUFU. While SHH was expressed by Purkinje cells in control cerebella to drive granule cell precursor proliferation, it was upregulated and misexpressed by granule cell precursors in SuFu-cko cerebella. 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 Purkinje cells (PC-SuFu-cko), respectively, using lineage-specific Cre recombinases. GC-SuFu-cko cerebella exhibited ectopic granule cells and delayed granule cell precursor differentiation whereas PC-SuFu-cko cerebella exhibited normal cerebellar patterning and differentiation. Together, our data demonstrate that GLI3R-mediated functions of SUFU are required for cell differentiation and cerebellar morphogenesis.

Cardiovascular Platform

TNFα IS CENTRAL TO THE AUGMENTED MYOGENIC RESPONSE OF SKELETAL MUSCLE RESISTANCE ARTERIES IN HEART FAILURE.

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Objective: Elevated peripheral resistance, a hallmark and determining factor for the progression of heart failure (HF), primarily results from the augmented myogenic response (MR) of resistance arteries (RA), which is the intrinsic property of RA to adapt their diameter to changes in pressure. In cerebral arteries from HF mice, tumor necrosis factor α (TNFα) plays a central role for the enhancement of the MR (through activation of sphingosine kinase-1). We hypothesized that TNFα is equally important for the augmentation of MRs in skeletal muscle RA and consequently contributes to the increased peripheral resistance in HF.
Methods: HF was induced in male C57/BL6 mice through surgical ligation of the left anterior descending coronary artery (LAD). 6-8wks post-ligation, cremaster muscle RA were isolated and cannulated on a pressure myograph.
Results: Elevations in transmural pressure (20-100mmHg in 20mmHg steps) induced MRs that were significantly augmented in RA from HF compared to sham-operated mice (n=7). MRs were not enhanced in RA from HF TNFα-/- mice (n=5). However, systemic treatment of LAD-ligated C57/BL6 mice with the TNFα scavenger etanercept (1mg/kg 2x/wk for 6wks post-ligation) did not block the HF-induced increase in MRs (n=7).
Conclusions: Our data confirms that the augmented MR in skeletal muscle RAs contributes to increased vascular resistance in HF. Results from TNFα-/- RAs indicate a mandatory role for this cytokine in this adaptive response. The fact that systemic TNFα sequestration can be fully compensated supports the concept of the MR as a redundantly organized mechanism and has important implications for therapeutic considerations.
(This study was funded by CIHR, NSERC, and HSFO)

BLOOD PRESSURE REACTIVITY TO PHYSICAL AND MENTAL STRESS IN ADOLESCENCE: THE ROLE OF SEX, PUBERTY STAGE AND VISCERAL AND WHOLE-BODY ADIPOSITY

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Blood pressure (BP) in children has been on the rise in recent years. This is, in part, explained by the increase of overweight and obesity in children. BP is higher in males than females throughout their reproductive lives, and adult BP and body composition develop during adolescence. Greater BP reactivity to and slower BP recovery from physical and mental challenges contributes to chronic BP elevation and hypertension target-organ damage. Relatively little is known about (1) sex differences in BP reactivity/recovery during early and late puberty and (2) the relationships of BP reactivity/recovery with visceral and whole-body adiposity. The aim of the present study was to examine these gaps in knowledge. Study participants (n=599, ages 12-18), recruited from the Saguenay-Lac St. Jean region of Quebec, underwent a 1-hour cardiovascular protocol during which BP was continuously recorded using a Finometer; the protocol included physical (i.e. standing for 10 min after laying down for 10 min) and mental (a 2-min math test) challenges. Visceral fat (VF) was quantified with magnetic resonance imaging and total body fat (TBF) was measured with bioimpedance. Our results showed that (1) BP reactivity to and BP recovery from both challenges were significantly greater and slower, respectively, in boys than girls (p=0.04-0.0009). (2) In boys, VF independently of TBF was positively related to the reactivity of BP to standing (p=.004), whereas in girls, TBF independently of VF was positively related to slowing down the recovery from standing (p=.003). All our results were independent of height and initial BP and were similar for systolic and diastolic BPs and during early and late puberty. These findings suggest important sex differences in cardiovascular reactivity to and recovery from stress and their relationship to various fat depots in adolescence.

ELUCIDATION OF THE PROTECTIVE MECHANISM OF A CRYSTALLIN B IN CARDIOMYOCYTES

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α-Crystallin B (cryAB) is the most abundant small heat shock protein in cardiomyocytes (CMs) where it has been shown to have potent anti-apoptotic properties. Studies show that ex vivo perfused hearts from transgenic mice that over-express cryAB tolerate ischemia/ reperfusion better, while cryAB null mouse a higher cell death rate following ischemia/reperfusion compared with wild-types. The mechanism by which cryAB prevents apoptosis has not been fully elucidated. We hypothesized that 1) cryAB levels will affect CM viability under oxidative stress conditions, and 2) cryAB regulation in CMs will attenuate apoptosis by binding and inhibiting caspases and/or Bax and cytochrome c proteins. Results: 1) Plasmids expressing shRNA targeting mouse cryAB mRNA or cryAB cDNA were used for Lentivirus transduction of neonatal CMs to knockdown and over-express cryAB, respectively. The protective properties of cryAB were assessed following exposure of CMs to 200 μM H2O2 for 16 hours using a colorimetric viability assay. 2) CryAB localization was determined using sub-cellular fractionation and its interactions with pro- apoptotic proteins were determined using co-immunoprecipitation. A significant decrease in viability was observed in WT CMs when cryAB was silenced when compared with WT CMs following exposure to H2O2 ($p < 0.001$). CryAB was found mainly in the cytosol in control conditions but mainly in the mitochondria following H2O2 exposure. Co-immunoprecipitation revealed a stronger physical interaction of cryAB with caspase 3 and cytochrome C in stressed cells when compared to controls. The interaction between cryAB and caspase 12 was similar in control and stressed cells. These findings validate the protective effects of cryAB in CMs following exposure to H2O2 oxidative stress. These protective effects are mediated by the translocation of cryAB from the cytosol to the mitochondria under conditions of stress. Furthermore, the interaction of cryAB with caspase 12 is passive and it does not contribute to its protective effects. Its interaction with caspase 3 and cytochrome C, however, is part of its protective mechanism following exposure to H2O2 in CMs.

Brain Research and Integrated Neurophysiology Platform

SYNERGISTIC EFFECTS OF STROKE AND ALZHEIMER'S PATHOLOGIES ON NEURONAL SURVIVAL OF ADULT BORN NEURONS 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. Progression of AD is advanced by risk factors for vascular cognitive impairment, such as stroke and diabetes. Our team has generated animal models to investigate the underlying pathological interactions between the risk factors and AD. Four groups of young adult male Sprague-Dawley rats were used in the study: sham controls, AD model, stroke model and the combined (AD&stroke) model. 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 a bilateral injections of Aβ25-35 into the lateral ventricles. It has been previously shown that the combined AD&stroke model presented with a synergistic increase in inflammation, especially in the hippocampal CA1 region and striatum, and in infarct size around the ischemic region. These pathologies also correlated with motor impairment and memory deficits. The present study examined adult neurogenesis in the dentate gyrus, a primary neurogenic region in the adult brain that is crucial for learning and memory. Immunohistochemical markers of new neurons BrdU, Doublecortin and NeuN were used to quantify changes in neurogenesis. The stroke or AD treatment alone, resulted in aberrant dendritic development and impaired neuronal maturation of adult-born neurons 3 weeks after the treatment. Combined treatment reduced neuronal survival by over 90% via a synergistic mechanism. This is the first evidence that a stroke in the striatum can persistently impinge on the normal dendritic development and neuronal maturation of the developing neurons in the dentate gyrus. Combined stroke&AD can further impact neuronal survival, 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. Lulu Gao is supported by the Novo Nordisk Studentship from Banting and Best Diabetes Center.)

A NOVEL 5-HT RECEPTOR SUBTYPE IN RODENT PREFRONTAL CORTEX: THE 5-HT5A RECEPTOR.

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The 5-HT5a receptor is the least well understood of the many serotonin (5-HT) receptor subtypes. In fact, the 5-HT5a receptor remains only provisionally classified in the 5-HT receptor family due to lack of functional evidence in native whole tissue. Here, we report the first functional characterization of the naturally-occurring 5-HT5a receptor in the rodent brain. Using whole cell recordings in brain slices of adult prefrontal cortex, we found a previously uncharacterized inhibitory 5-HT current that was suppressed by the selective 5-HT5a antagonist, SB 699 551 (10 μM; 20 min). This current was present in rats and in two strains of mice, however, was completely absent in 5-HT5a-/- mice. Electrophysiological characterization revealed that this current is inwardly-rectifying with a reversal potential near the K+ ion equilibrium potential and was suppressed by K+ channel inhibitors, Ba+ and Tertiapin-Q, suggesting that this current is mediated by G-protein inwardly rectifying K+ channels. Finally, in 5-HT5a-/- mice we observed a dramatic degree of plasticity the 5-HT1A receptor responses. Specifically, 5-HT5a-/- mice display increase in the amplitude of the 5-HT1A receptor current, a closely related receptor known to be involved in the pathology and treatment of psychiatric disorder. These findings represent the first functional characterization of the 5-HT5a in native brain tissue and suggest an important role of the 5-HT5a receptor in prefrontal serotonin signaling.
This research was supported by an NSERC Discovery Grant (EL) and a CHIR Banting and Best Doctoral Award (NG).

THE ROLE OF ΔGABAARS IN COGNITION AND SYNAPTIC PLASTICITY.

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Extrasynaptic γ-aminobutyric acid subtype A receptors (GABAARs) are robustly expressed in the hippocampus and dentate gyrus and generate a persistent inhibitory current. Extrasynaptic GABAARs containing the δ subunit (δGABAARs) are responsive to drugs (such as anesthetics and ethanol) and hormonal states (pregnancy, puberty and stress), but their exact function remains to be elucidated. To determine the functional role of δGABAARs, we compared cognition and synaptic plasticity, a molecular substrate of learning and memory, in wildtype (WT) and δGABAAR knockout (Gabrd-/-) mice. To ascertain the effects of increased δGABAAR function, we also examined the effects of δGABAAR-preferring agonist 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP) on these mice. WT and Gabrd-/- mice performed similarly on most behavioural tests. However, WT and Gabrd-/- mice performed differently on the object recognition ($t=1.89, df=29, p<.05$) and object displacement tasks ($t=1.84, df=31, p<.05$). Gabrd-/- mice demonstrated impaired performance on the object displacement task but surprisingly improved performance on the object recognition task. WT and Gabrd-/- mice also demonstrated similar plasticity in the dentate gyrus. In contrast, selective activation of δGABAARs with THIP impaired contextual fear conditioning ($F(1,107)=10.86$, $p<.05$), tended to impair water maze performance and strongly attenuated plasticity in WT but not Gabrd-/- mice. These results show that the role of the δGABAAR in cognition and plasticity is normally subtle but becomes significant when the receptor is strongly activated. δGABAARs thus play a dynamic, bidirectional role in cognition that depends on the pharmacological or physiological state.

Poster Abstracts

1. ENERGETIC DYSHOMEOSTASIS IN APP-TRANSGENIC TGCRND8 MICE.

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TgCRND8 mice express a double mutant (K670N/M671L + V717F) human amyloid precursor protein (APP) transgene. We previously reported that these mice develop progressive object memory impairment from 8 weeks and β-amyloid plaques by 15 weeks of age (Francis et al., Neurobiol. Aging, Epub May 4th 2010). To determine how mutant APP-expression might affect mitochondrial function, we assessed activities of complex I+III and IV of the electron transport chain, as well as those of α-ketoglutarate dehydrogenase (KGH) and pyruvate dehydrogenase (PDH). Pre-plaque, behaviourally impaired TgCRND8 mice exhibited increased complex I+III activity in cortex. In contrast, complex I+III activities were reduced by 45% in one-year-old TgCRND8 mice. Complex IV, KGH and PDH activity levels were unaffected. The dissociation between effects of the APP-transgene on complex I+III and other key mitochondrial enzymes suggests that Aβ exposure may not simply alter mitochondrial number. This was confirmed by Western Blot analysis of citrate synthase expression. The late life reduction in complex I+III activity coincided with reduced expression of NDUFB8, a mitochondria-encoded subunit integral to the assembly and function of complex I. In contrast the increased activity in young mice was not reflected in altered enzyme expression. Moreover, fluctuations in oxidative phosphorylation may not be due to changes in mitochondrial membrane lipids. Cardiolipin is an inner membrane phospholipid that is crucial to the biogenesis and function of the electron transport chain. We measured cardiolipin by lipid extraction and chromatography, and found no differences in its acyl-chain composition or availability. To determine whether changes in oxidative phosphorylation are associated with altered mitochondrial output, we examined tissue levels of high-energy phosphate donors by reverse phase HPLC and UV analysis. TgCRND8 mice exhibit widespread increases in phospho-creatine (P-Cr) levels in both pre-plaque and advanced plaque stage animals. Tissue ATP levels tracked with complex I+III activity in the cortex of pre-plaque mice. However, ATP content did not fall with the 45% reduction in complex I+III activity in one-year old mice. Increases in P-Cr may buffer ATP thereby mitigating the impact of decreased complex I+III activity in aged TgCRND8 mice. Indeed, ATP was only increased in brain regions that also exhibit elevated creatine levels. Our results suggest an early disruption of energetic homeostasis in TgCRND8 mice, and highlight the likely role of the P-Cr/Cr buffer system in maintaining ATP levels.

2.. MITOCHONDRIAL SIRTUIN SIRT3: A NOVEL CYTOPROTECTIVE FACTOR?

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Ageing brings a greater risk of debilitating conditions, which often arise from an overall reduction of metabolic efficiency and the increased genesis of reactive oxygen species (ROS). Impaired mitochondrial function has been hypothesized to contribute significantly to age-related decline. Identifying means by which mitochondrial activity can be strengthened, and/or less ROS generated, is a common theme in many age-related and research programs. Recent work suggests that a member of the Sirtuin protein family, namely the Sirtuin, SIRT3, may be able to directly or indirectly influence both of these processes. SIRT3 is a descended of a yeast ancestral gene known to possess longevity-enhancing activity, and the SIRT3 gene resides in a region of the human genome known to contain longevity-enhancing genes. SIRT3 activity is activated by the red wine resveratrol, and its expression is induced by longevity-enhancing diets. These observations provide a strong rationale for further investigation of whether SIRT3 does, indeed, possess cytoprotective and longevity-enhancing potential. To begin addressing whether SIRT3 possesses cytoprotective factors I demonstrated that elevating SIRT3 expression in cultured cells partially decreases their resting mitochondrial membrane potential- which has been demonstrated to improve mitochondrial efficiency. Additionally I have shown that SIRT3 decreases basal ROS levels, and attenuates the increase in ROS levels caused by Glucose Deprivation (GD) in cultured cell lines. Importantly, I also have found that cells over-expressing SIRT3 are protected from both GD and Hydrogen Peroxide (H2O2) challenges. To investigate whether SIRT3 may play a role in neuroprotection using quantitative real time PCR I compared its expression to a known neuroprotective sirtuin, SIRT1, in the murine brain. My results show that SIRT3 and SIRT1 mRNA levels are robustly expressed in different brain regions including hippocampus, cortex, striatum, and cerebellum throughout development, with SIRT3 being expressed at significantly higher levels in each brain region. The fact that my results show that SIRT3 possesses robust cytoprotective ability, and is highly expressed in the brain demonstrates that it may be a potential neuroprotective target in age related decline.

3. MECHANISM OF CHEMICAL TRANSMISSION BETWEEN A GLIAL CELL AND ITS ASSOCIATED NEURON IN THE CHICK DORSAL ROOT GANGLION.

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Glial cells are closely associated with chemical synapses throughout the nervous system. Although their structurally supportive roles in processes such as axon myelination and synaptogenesis are relatively well characterized, less is known about the involvement of glial cells in neuronal function. In this study we explore the mechanism of chemical transmission between a glial cell and its associated neuron in the chick dorsal root ganglion (DRG). DRG neurons were gently dissociated to retain connections with their ensheathing satellite glial cells (SGCs). The patch clamp technique was used to record ion currents from cells and pharmacological agents were applied to dissect the signaling pathways. We found that BzATP, a P2X7 receptor agonist, puffed onto a SGC can generate a dose duration-dependent increase in inward current in the ensheathed neuron. The purinergic antagonist suramin inhibited the drug's effect. BzATP puffed directly onto a fully isolated neuron had little or no direct effect on DRG neuron current fluctuations, suggesting that the neuronal response can be attributed to SGC secretion. This was verified by the finding that transmission was inhibited in the intact structure in the presence of nicotinic antagonists d-tubocurarine and pancuronium bromide to block neuronal acetylcholine (ACh) receptors. Our results suggest that activation of purinergic receptors on SGCs triggers the secretion of ACh which activates the associated DRG neuron. (This study was funded by the Canadian Institute for Health Research.)

4. NOVEL CALCIUM BINDING PROTEIN CALTUBIN MEDIATED REGULATION OF VOLTAGE GATED CALCIUM CHANNELS L. STAGNALIS AND MAMMALIAN TSA-201 CELLS.

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Calcium binding proteins are essential for the regulation of voltage gated calcium channels (VGCC), irregularities in regulation have been attributed to many pathologies such as episodic ataxia and spinocerebellar ataxia. Here it is shown that novel Calcium binding protein Caltubin under siRNA knockdown shows a alteration in the IpeakBa2+, IsusBa2+, GpeakBa2+ GsusBa2+, ErevpeakBa2+ ErevsusBa2+, biophysical properties in invertebrate Lymnaea stagnalis. Also, when Caltubin is expressed with CaV2.1 channels with β2 subunits in mammalian TSA-201 cells IpeakBa2+, GpeakBa2+ , ErevpeakBa2+ show an increase. These findings 1.) make Caltubin an essential regulator of VGCC and by extension the myriad of physiological processes 2.) suggests Caltubin does regulate VGCC in mammalian system 3.) provides a potential therapeutic target for improper Calcium channel regulation 4.) regulation of calcium channel by Caltubin is independent of invertebrate or vertebrate environment and opens the possibility of study of other proteins restricted before to only invertebrate study.

5. ANTIFIBRINOLYTIC DRUGS INHIBIT GLYCINE RECEPTORS IN MOUSE NEURONS.

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Antifibrinolytic agents such as tranexamic acid (TXA), epsilon aminocaproic acid (EACA) and aprotinin are widely used to reduce blood loss during surgery (CMAJ 2009 180: 183-193). Both TXA and EACA have proconvulsant properties in humans and animal models (J Neurol 1999;246:843; JAMA. 1978;240:2468) and TXA is associated with a marked increase in the incidence of post-operative seizures (Anesth Analg 2010 111: 580-581). The mechanisms underlying these seizures are unknown. Receptors for the inhibitory neurotransmitter glycine (GlyRs) are widely expressed in the central nervous system and pharmacological inhibition of GlyR function has been associated with seizures (J Neurophysiol, 2002 87: 1515–1525). Notably, TXA and EACA are structural analogues of glycine (Gly) which suggests that these drugs modulate GlyR activity. Here we tested the hypothesis that clinically relevant concentrations of TXA and EACA inhibit GlyRs whereas aprotinin, which is not a structural analogue of Gly, does not. Method: Whole-cell voltage clamp techniques were used to record Gly-evoked currents from cortical and spinal neurons of embryonic mice grown in culture. Results: TXA and EACA reversibly inhibited GlyRs with a half-maximal inhibitory concentration (IC50) of 1.12 ± 0.05 mM and 12.3 ± 0.9 mM, respectively, whereas aprotinin had no effect. TXA and EACA shifted the Gly concentration-response curve to the right and did not reduce the maximum response, suggesting that these drugs act as competitive antagonists at the GlyRs. Consistent with this interpretation, the Schild regression plot for TXA-mediated inhibition of GlyRs revealed a slope of 0.83 ± 0.07. This study provides the first evidence that TXA and EACA, at clinically relevant concentrations inhibit GlyRs. In addition, these drugs act as competitive antagonists, a property that may contribute to their association with postoperative seizures seen in patients.

This work was supported by a CIHR grant to AT

6. THE FATTY ACID PALMITATE DISRUPTS NORMAL CIRCADIAN CLOCK GENE TRANSCRIPTION IN HYPOTHALAMIC NEURONS.

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The hypothalamus is largely considered the master regulator of energy homeostasis. Specifically, it has been demonstrated that maintenance of cellular function is essential for energy balance and metabolism. At the cellular level, AMP-activated protein kinase (AMPK), acts as a potent energy sensor, having been implicated in the regulation of glucose uptake and β-oxidation. Excessive nutrient levels, as seen in pathophysiological conditions like obesity, can lead to metabolic disturbances. Moreover, recent discoveries have linked metabolic dysfunction with circadian dysregulation. Traditionally associated with sleep-wake cycles, circadian rhythms are endogenous cycles found in nearly all organisms and cells and are responsible for regulating a myriad of physiological processes, including metabolism and energy homeostasis. Obesity has been shown to alter circadian gene transcript levels and conversely, atypical circadian rhythms lead to obese phenotypes. However, the molecular mechanisms underlying this phenomenon are not completely understood and difficult to study in vivo. To address this issue, our lab has generated and characterised a subset of immortalized, clonal, mouse hypothalamic neurons that demonstrate a strong rhythmicity and express essential feeding related neuropeptides. Therefore these neurons present a potential cellular model with which to study the inter-relationship between circadian rhythms and metabolism. We hypothesize that the saturated free fatty acid, palmitate leads to circadian dysregulation in vitro. Using the neuronal cell models, mHypoE-44 and mHypoE-37, we are currently examining the effects of palmitate on the mRNA levels of key circadian molecular components, Bmal1, Clock, Per2, and Rev-erbβ. Furthermore, with the use of aminoimidazole carboxamide ribonucleotide (AICAR), an AMPK agonist, we are investigating whether changes in AMPK activity lead to alterations in key clock genes. Preliminary qRT-PCR data suggests prolonged exposure to elevated levels of palmitate (0.3μM) changes the rhythmicity of expression levels of these clock genes. By further understanding the effects of nutrient excess at the cellular level, we will be able to further delineate the complex mechanisms involved in the development of obesity. We acknowledge funding from CIHR, NSERC, CFI, and the CRC Program.

7. INTENSITY OF THE THERMAL GRILL ILLUSION DEPENDS ON CONFIGURATION OF GRILL ELEMENTS.

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The thermal grill (TG) illusion of pain (TGI) occurs in response to simultaneous application of interlaced warm (40 °C) and cool (20 °C) bars. The illusion is proposed to reflect the altered balance of the activity of innocuous cool and cold responsive nociceptive (termed HPC cells) spinal neurons leading to less cool-induced inhibition of pain. Previous studies of the TGI used multiple alternating warm and cool bars delivered to the hand and forearm. In this study, we evaluated the effect of varying the spatial arrangement of warm vs. cool bars on perceived pain and unpleasantness at the forearm and calf. Thermal stimuli (duration, 30s) were produced by an array of 5 Peltier-controlled bars, in 4 different arrangements as follows: two control conditions - all bars 20 °C, all bars 40 °C, and two TG conditions: center bar 20 °C /outer bars 40 °C (TG1), and center bar 40 °C /outer bars 20 °C (TG2), presented in randomized order in 26 male subjects. On consecutive runs, subjects continuously rated either pain or unpleasantness intensity on an electronic visual analogue scale. Six subjects did not perceive pain or unpleasantness to any of the four conditions at both body sites, and were excluded from subsequent analyses. Statistical analysis revealed a significant effect of configuration ($p < 0.002$ for pain, $p < 0.039$ for unpleasantness). Interestingly and unexpectedly most of the 12 subjects reported that the TG1 configuration was more painful than the TG2 configuration ($p < 0.014$). Furthermore, the mean intensity of pain ($p < 0.004$) and unpleasantness ($p < 0.004$) evoked by TG1 was higher in the calf vs. the forearm. These findings suggesting that a single cool bar surrounded by warm bars produces a more intense TGI compared to the opposite configuration may possibly be explained by a reduced cool-induced inhibition of the pain (HPC) pathway in the former configuration. Additional studies are required to elucidate the mechanisms underlying these phenomena. Acknowledgments: NSERC/CIHR CHRP Grant 350980

8. FAST FIRING BASKET CELLS CONTRIBUTE TO THETA RHYTHM GENERATION IN MODEL HIPPOCAMPAL CA1 NETWORKS.

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Theta rhythms, 3-12 Hz oscillations recorded in the hippocampus of all mammals studied to date[1], are thought to play a lead role in spatial navigation and episodic memory. These rhythms are recorded from the hippocampus during R.E.M. sleep and “voluntary”

behaviour such as exploration, and are critically involved in the timing of place cell firing[1]. Although these oscillations have been heavily studied, the mechanism(s) responsible for the generation of these rhythms remains unknown. A classic theta model predicts that pacemaker neurons in the medial septum drive hippocampal rhythms[1], but recent research using an intact hippocampus preparation in vitro suggests that the CA1 hippocampal region possesses the necessary circuitry to generate robust intrinsic theta rhythms[2]. To determine the mechanism(s) underlying the generation of these CA1 hippocampal theta rhythms, we created a mathematical network model.
Our mathematical network model is composed of four types of cells: pyramidal cells, fast-spiking parvalbumin-positive basket cells (PV+BCs), slow-spiking cholecystokinin-positive basket cells (CCK+BCs), and oriens – lacunosum-moleculare (O-LM) interneurons. The network configuration is based on experimental data of known connectivities, representing the intrahippocampal connections among the chosen cell types, and each cell type is represented by a single-compartment conductance-based model.
Our network model produces robust theta rhythms in accordance with the experimental data. Interestingly, we find that inhibitory input imposed on pyramidal cells from the PV+BCs are key in producing theta rhythms. This finding is surprising, as research has focused on the role of PV+BCs in faster gamma rhythms (20-100 Hz). Synaptic constraints from the experimental data applied to the network model will determine the applicability of the underlying network model mechanism to the biological system. (This study was funded by the Canadian Institutes of Health Research)

9. NON-MOTOR BEHAVIOURS AND STRIATAL MONOAMINE METABOLITES ARE CHANGED IN DJ-1-DEFICIENT MALE MICE.

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Mutations in the DJ-1 gene result in a loss of functional DJ-1 protein and cause early-onset familial Parkinson's disease (PD). DJ-1-knockout (DJ-1-KO) mice exhibit hypersensitivity to MPTP, a dopaminergic neurotoxin, as well as progressive disruption of gait and impaired corticostriatal and hippocampal synaptic activity. Thus, it appears that DJ-1 is involved in the function of brain regions beyond the dopaminergic nigrostriatal neurons that are targeted in PD. In this study, we tested the DJ-1-KO mice in a battery of cognitive, neuromotor and affective behavioural paradigms. Striatal monoamines and their metabolites were assessed with high performance liquid chromatography. We found the DJ-1-KO mice had impaired performances in the Morris water maze and the Barnes maze, both of which test for spatial memory. Non-spatial object recognition memory was also impaired in the DJ-1-KO mice. In a test of forelimb coordination that has been shown to be sensitive to disrupted nigrostriatal dopamine, only male DJ-1-KO mice exhibited impairments. Similarly, in a test of behavioural despair, male DJ-1-KO mice exhibited increased immobility, a measure that is inversely correlated with synaptic noradrenaline turnover. The DJ-1-KO male mice were also observed to have a reduction in the exploration of open quadrants in the zero-maze, a measure of anxiety that is sensitive to serotonin transmission. Our neurochemical analysis showed that male DJ-1-KO mice had an increase in striatal dopamine, homovanillic acid and normetanephrine content. Serotonin and norepinephrine content were elevated in both male and female striatum. The regulation of sex hormones may influence gender differences in behaviours and PD susceptibility. DJ-1 was reported to be a positive regulator of the androgen receptor. The loss of DJ-1 functions may disrupt normal androgen receptor signaling, leading to the male phenotypes observed in the DJ-1-KO mice.

10. EXPRESSION, PHOSPHORYLATION, AND GLYCOSYLATION LEVELS OF CNS PROTEINS IN AVERSIVE OPERANT CONDITIONING ASSOCIATED MEMORY IN LYMNAEA STAGNALIS.

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Long-term memory formation requires expression and post-translational modification of many proteins. Understanding the temporal and spatial regulatory pattern of these proteins is fundamental to decoding the molecular basis of learning and memory. We characterized changes in expression, phosphorylation and glycosylation of CNS proteins after operant conditioning in the pond snail Lymnaea stagnalis. The phosphorylation and the glycosylation levels of proteins, measured by the ratio of Pro-Q Diamond (phosphoproteins) or Pro-Q Emerald (glycoproteins) versus SYPRO-Ruby (total proteins) signals, increased during memory formation. The regulated phosphoproteins are associated with cytoskeleton, glutamine cycle, energy metabolism, G protein signaling, neurotransmitter release regulation, iron transport, protein synthesis, and cell division. We further confirmed that phosphorylation of actin, one of the phosphoprotein identified, increased during memory formation. To identify proteins whose expression levels changed in long-term memory formation we used two-dimensional difference gel electrophoresis followed by mass spectrometry. Proteins whose modulation of phosphorylation might be involved in learning and memory were identified by mass spectrometry (MS) and are associated with cytoskeleton, glutamine cycle, energy metabolism, G protein signaling, neurotransmitter

release regulation, iron transport, protein synthesis, and cell division. Phosphorylation of actin, increased during memory formation. To identify proteins whose expression levels changed in long-term memory formation we used two-dimensional difference gel electrophoresis followed by MS. The up-regulated proteins are mostly associated with lipoprotein and cholesterol metabolism, protein synthesis and degradation, cytoskeleton, nucleic acid synthesis, energy supply. The down-regulated proteins are enzymes of aspartic acid metabolism involved in regulation of protein synthesis. Our proteomic analyses have revealed a number of candidate proteins associated with memory formation. Using Spider PPI Networking profile program we inferred a bioinformatics interaction network that links these proteins. Further investigation into the complex signaling network that they are part of as well as their individual functions will advance our understanding of memory formation and consolidation. L. Silverman-Gavrila was supported by Fellowship from the Heart and Stroke Foundation of Canada and NSERC and a CIHR Short-term Travel Grant. This work was funded by operating grants to M. Charlton (Canadian Institute of Health Research MOP-82827) and Z-P. Feng (MOP 62738).

11. SPATIAL EXTENT OF B-COHERENT ACTIVITY IN THE STN AND SNR OF PARKINSON'S DISEASE PATIENTS

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Objective: To measure the spatial extent of β-coherent activity in the STN and SNr of PD patients ON/OFF levodopa using dual microelectrode LFP recordings.
Hypothesis: A progressive attenuation in β power from dorsal to ventral STN is expected in patients OFF levodopa. Moreover, it is anticipated that coherence between β-LFPs of STN and SNr will be observed.
Methods: The data was collected from 4 human subjects undergoing DBS-STN implantation surgery. Recordings where obtained from trajectories passing dorsoventrally through STN using two microelectrodes initially 600 μm apart and digitally sampled at 15 kHz. Once the microelectrodes entered the dorsal STN, one of the two was held stationary, while the other one advanced toward the ventral border of STN and then into SNr over a distance of 4-6 mm.
Results: We confirmed a progressive attenuation in β power as electrodes were guided from dorsal to ventral STN in patients OFF levodopa. However, significant β-LFP coherence was still found across the dorsoventral extent of STN. Furthermore, a significant coherence was found between β-LFPs located in dorsal STN and dorsal SNr.
Conclusions: These preliminary data showing LFP synchrony within STN suggests the whole nucleus is entrained within the beta band in PD patients OFF meds. The finding of coherence between STN and SNr, suggests that β oscillations are also at the output of the basal ganglia.

12. GLUCOSE ADMINISTRATION DOES NOT PREVENT MORTALITY IN CONVULSING HYPOGLYCEMIC JUVENILE DIABETIC RATS.

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Human juvenile diabetics 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 is systematically investigating the impact of a severe hypoglycemic episode in juvenile diabetic animals to assess pathophysiology and potential treatment strategies. Postnatal 21 day old male Sprague Dawley rats were treated with streptozotocin (STZ), a β cell-specific toxin, to produce the type 1 diabetes phenotype. Animals were randomly separated into two groups, control and STZ-treated, and were fasted overnight (16 hours) before intraperitoneal (ip) injection of STZ or citrate buffer (control). The optimum dose for the diabetogenic response to STZ (80 mg/kg) was determined using different concentrations of STZ (60-80mg/kg) in fasted and non-fasted animals. Seven days after a single dose of 80 mg/kg STZ in overnight fasted animals, 91% became diabetic. Diabetic animals demonstrated elevated blood glucose levels (above 280 mg/dl) within 2-7 days and delayed weight gain, both factors which persisted up to 2 months of age. At 28 days, the diabetic animals were fasted overnight and treated with insulin, 10-15 u/kg ip, and were monitored for seizure-like activity. Repeated behavioral seizures were observed when blood glucose levels dropped below 50 mg/dl. Glucose administration alone even when repeated, was not usually successful in reviving seizing animals. Treatment ip with 500ug of diazepam, 5mg of phenytoin and 0.5ml of 25% glucose stopped seizures and prevented death in 58% of rats (N=27). There was no mortality in animals made hypoglycemic who did not exhibit seizure behaviour. These data show that glucose administration alone in hypoglycemic juvenile diabetic animals does not prevent seizures which usually are fatal, unless stopped by anticonvulsant therapy.

13. IDENTIFICATION OF THE ROLE OF C/EBP IN NEURITE REGENERATION FOLLOWING MICROARRAY ANALYSIS OF A L. STAGNALIS CNS INJURY MODEL.

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Neuronal regeneration in the adult mammalian central nervous system (CNS) is severely compromised due to

the presence of extrinsic inhibitory signals and a reduced intrinsic regenerative capacity. In contrast, the CNS of adult Lymnaea stagnalis (L. stagnalis), a freshwater pond snail, is capable of spontaneous regeneration following neuronal injury. Thus, L. stagnalis has served as an animal model to study the cellular mechanisms that are involved in neuronal regeneration. However, the usage of this model has been limited due to a lack of molecular tools. We have recently conducted a partial neuronal transcriptome sequencing project and reported over 10,000 EST sequences which allowed us to develop a large-scale high throughput microarray analysis. To identify genes that are involved in the robust regenerative capacity observed in L. stagnalis, we designed the first gene chip covering ~15, 000 L. stagnalis CNS EST sequences. We conducted microarray analysis to compare the gene expression profiles of sham-operated (control) and crush-operated (regenerative model) central ganglia of adult L. stagnalis. The expression levels of 348 genes were found to be significantly altered ($p < 0.05$) following nerve injury. We further carried out real-time reverse transcription PCR (qPCR) analysis and confirmed that CCAAT enhancer binding protein (C/EBP) was up-regulated following nerve injury in a time-dependent manner. In order to test the role of C/EBP in regeneration, C/EBP siRNA was applied following axotomy of cultured Lymnaea PeA neurons. Knockdown of C/EBP following axotomy prevented extension of the distal axon, suggesting the involvement of C/EBP in neuronal regeneration may be mediated by its local effects at the growth cone. This is the first high-throughput microarray study in L. stagnalis, and provides the first evidence for the involvement of local C/EBP in neuronal regeneration. Our study demonstrates the usefulness of the large-scale gene profiling approach to study the molecular mechanisms underlying the intrinsic regenerative capacity of adult CNS neurons in this invaluable invertebrate model.
(This study was funded by the Canadian Institute for Health Research, and the student is an OGS recipient)

14. INCREASING CRTC (CREB REGULATED TRANSCRIPTION COACTIVATOR) LEVELS IN THE DORSAL HIPPOCAMPUS IS SUFFICIENT TO INDUCE CONTEXTUAL FEAR MEMORY AND DISCRIMINATION.

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Previous research has established that the CREB (cAMP/Ca2+ 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 a strong contextual fear memory under weak training conditions that do not support contextual memory formation 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. This work was supported by grants from the Canadian Institutes of Health Research (CIHR; MOP-74650, SAJ; MOP-86762, PWF), EJLB Foundation (SAJ) and Natural Science and Engineering Research Council (NSERC; SAJ). MJS received support from a Restracomp Fellowship (Hospital for Sick Children) and a CIHR Frederick Banting and Charles Best Canada Graduate Scholarships Doctoral Award.

15. UBIQUITOUS RESTORATION OF MECP2 IN A MOUSE MODEL OF RETT SYNDROME LEADS TO PHENOTYPIC IMPROVEMNTS.

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Rett syndrome (RTT) is a neurodevelopmental disorder affecting 10,000 to 15,000 live female births and is one of the leading causes of mental retardation in females. Defects of the methyl-CpG-binding protein 2 (MeCP2) gene located on the X chromosome has been identified as a major cause of RTT. MeCP2 is expressed in all cells but is most abundantly expressed in neurons. MeCP2 is a nuclear protein that specifically binds to methylated DNA which then represses gene expression through the recruitment of histone deacetylases. Importantly, recent studies have shown that deficits caused by MeCP2 deficiency are not irreversible. Therefore, we hypothesized that restoration of MeCP2 to the brain will correct deficits associated wit RTT. Results: 1) A RTT rescue model was produced by crossing RTT mice, having a loxP flanked stop cassette, with mice that consist of an inducible estrogen

receptor attached Cre-recombinase system. 2) Weekly tamoxifen (estrogen receptor antagonist) injections were given to the rescue mice, RTT mice, and wild-type mice. 3) The mice were assessed using a gross behavioural scoring system, open field ambulation test, accelerating rota-rod test, and nesting-behaviour test. 4) Mouse brains were collected following heparin-saline perfusion. Western blotting has confirmed a restoration of MeCP2 protein levels in the rescue mice brains. The rescue mice showed a significant improvement in their gross behaviour compared to RTT mice that had no MeCP2 re-expression. Furthermore, reactivation of MeCP2 significantly extended the life span of the rescue mice (p-value < 0.05). There is a modest improvement in the nesting behaviour of the rescue mice, however, no significant improvements were observed in the open field and rota-rod performances compared to RTT and Wt mice. These findings suggests that restoration of MeCP2 in a mouse model of RTT leads to improved gross behaviour and extended life span, however, the rescue is incomplete.

16. 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 10μM 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.

17. A NOVEL LYMNAEA PROTEIN ESSENTIAL FOR CENTRAL NEURON REGENERATION REGULATES LOCAL EXPRESSION OF MICROTUBULES.

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Neuronal regeneration involves multiple mechanisms, including timely manipulation of the cytoskeleton and appropriate intracellular Ca2+ dependent responses. In response to growth or regenerative cues, local microtubule protein manipulation directly influences neurite behaviour. LCaBP is a novel Lymnaea protein containing putative EF-hands ubiquitously expressed in central neurons of the animal. The transcripts of LCaBP are localized in various regions of cells, including neurites and growth cones. To investigate the role of local LCaBP transcripts in growth cones, we studied isolated neurite behaviour by severing the communication between the soma and growth cones of cells in vitro via either mechanical transaction or pharmaceutical block of axonoplasmic transport. We discovered that only cells with adequate levels of LCaBP were capable of neuronal outgrowth and regeneration under these conditions. Since the process of neurite extension and regeneration are highly dependent on cytoskeletal manipulation, we tested the potential effect of LCaBP on the expression of actin and tubulin. LCaBP knockdown using target gene silencing approaches reduced expression of tubulin protein, without affecting actin level. Using immunohistochemistry and confocal microscopy, we demonstrated that LCaBP is specifically colocalized with tubulin in neurites and growth cones of cultured cells. Our co-immunoprecipitation assay revealed that LCaBP directly interacts with β-tubulin. Our data suggest that LCaBP is crucial for neurite outgrowth and regeneration in the central neurons of Lymnaea and that these effects are mediated, at least in part, via direct manipulation of microtubulin expression level.
(This study was funded by a CIHR grant to ZPF (MOP151437) and a NSERC-CGS to NN)

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

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Rett Syndrome (RTT) is a neurodevelopmental disorder caused predominantly by mutations of the gene encoding the epigenetic factor Methyl-CPG-Binding Protein 2 (MeCP2). It is the most common monogenetic cause of mental retardation in females worldwide, displaying a host of neurological impairments with seizures being typically ranked as the co-morbidity that most significantly affects their quality of life. The seizures are generally poorly controlled by current therapies with classic anti-convulsive drugs. Thus, there is a need to screen for new drugs that will show effective action towards seizures in the MeCP2-deficient brain. A mouse model of RTT has been generated displaying abnormal spike wave discharges throughout their cortex. The neurochemical deficits responsible for these seizures are unknown but one system that is normally regulated by MeCP2 that has been speculated to play a key role in Rett syndrome pathogenesis is histone deacetylase-6 (HDAC6). Normally, MeCP2 attenuates the expression of this system, which when overactive inhibits critical factors involved in brain maturation such as Brain-Derived Neurotrophic Factor (BDNF). In fact, diminished BDNF levels are observed in the MeCP2-deficient brain, and enhancing BDNF expression in MeCP2-deficient mice modestly improves their Rett-like behavior. Given this, we hypothesize that partially inhibiting HDAC6 in the MeCP2-deficient brain will improve behavioural and neurophysiological activity. Results: 1) RTT model mice and Wild-type mice electroencephalogram (EEG) activity and behavioural performance was assessed. 2) Mice were treated with either vehicle (0.9% saline) or a novel HDAC6 inhibitor for four weeks. 3) EEG and behavioural performance was re-assessed. Preliminary data shows a significant decrease in spike wave discharge incidence in RTT mice following HDAC6 inhibitor treatment ($p<0.01$, $n=4$). RTT mice also demonstrated a significant improvement in the Rotating Rod behavioural test ($p<0.05$, $n=8$). These findings seem to suggest that HDAC6 inhibition offers some therapeutic effect in a mouse model of RTT.

19. ADULT HIPPOCAMPAL NEUROGENESIS AND MEMORY INTERFERENCE.

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The hippocampus is involved in the formation of distinct, contextually-rich memories and when its function is impaired memories become more susceptible to interfering influences. Evidence suggests that discrete neuronal representations within the dentate gyrus allow memories to be readily distinguished from one another, thus minimizing the effect of competing memories during selective recall. Adult neurogenesis is a unique feature of the dentate gyrus and is known to contribute to normal hippocampal function. We therefore hypothesized that neurogenesis plays a central role in the modulation of interfering influences during learning and memory. Methods: adult neurogenesis in rats was alternatively suppressed using low-dose irradiation, or enhanced by allowing the rats to engage in running. Some rats were subjected to both treatments. The rats were then trained in a visual discrimination task under conditions of high or low interference. Results: neurogenesis in irradiated rats was reduced and the animals exhibited a concordant increase in their susceptibility to memory interference, resulting in loss of memory for the previously learned discrimination. Learning and memory retention of the discrimination response under low interference conditions were unaffected. Irradiated rats that engaged in running activity exhibited increased neuronal growth and protection from memory impairment under high interference conditions. Conclusion: our results suggest that adult-born hippocampal neurons play a role in differentiating between conflicting, context-dependent memories, and provide further evidence of the importance of neurogenesis in hippocampus-sensitive memory tasks.

20. A SODIUM LEAK CURRENT REGULATES PACEMAKER ACTIVITY OF ADULT CENTRAL PATTERN GENERATOR NEURONS IN LYMNAEA STAGNALIS.

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The resting membrane potential of the pacemaker neurons is one of the essential mechanisms underlying rhythm generation. In this study, we described the biophysical properties of an uncharacterized channel (U-type channel) and investigated the role of the channel in the rhythmic activity of a respiratory pacemaker neuron and the respiratory behaviour in adult freshwater snail Lymnaea stagnalis. Our results show that the channel conducts an inward leak current carried by Na+ (ILeak-Na). The ILeak-Na contributed to the resting membrane potential and was required for maintaining rhythmic action potential bursting activity of the identified pacemaker RPeD1 neurons. Partial knockdown of the U-type channel suppressed the aerial respiratory behaviour of the adult snail in vivo. These findings identified the Na+ leak conductance via the U-type channel, likely a NALCN-like channel, as one of the fundamental mechanisms regulating rhythm activity of pacemaker neurons and respiratory behaviour in adult animals. (The work was supported by an operating grant to ZPF from National Sciences and Engineering Research Council of Canada (NSERC-249962-09). TZL is a recipient

of a Canadian Graduate Studentship (CGS-MSc) of NSERC. ZPF holds a New Investigator Award from the Heart and Stroke Foundation of Canada.)

21. A SUPERADDITIVE INTERACTION BETWEEN INFLAMMATION AND ETOMIDATE FOR MEMORY BLOCKADE IN MICE.

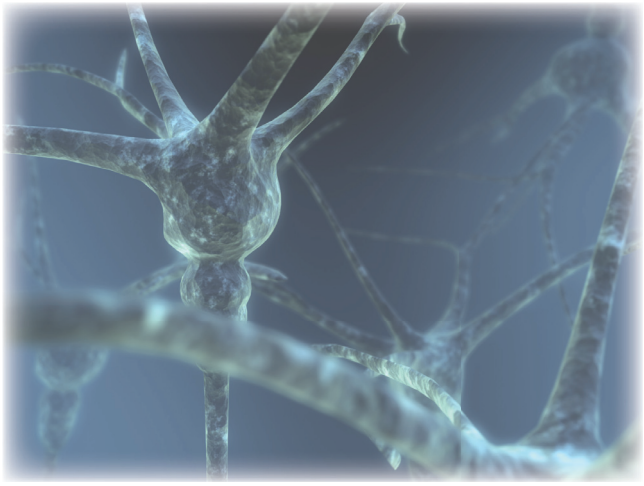
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Introduction: Seriously ill patients typically require lower doses of general anesthetics (1); however, the underlying reasons for the reduced anesthetic requirements have not been clearly elucidated. A major concern associate with the use of lower doses of general anesthetics is that patients will experience the explicit recall of surgical events and “intraoperative awareness” (2). It is suspected that general anesthetics and systemic inflammation may cause memory deficits in animal models (3). The goal of the present study was to determine whether systemic inflammation and etomidate interact to modify learning and memory. Methods: All experiments were approved by local ethics committee. Memory performance in 3-4 month old, male mice was assessed with contextual fear conditioning assay. The endotoxin lipopolysaccharide (LPS; 125 µg/kg, i.p.) was used to trigger systemic inflammation. Three hours prior to contextual conditioning, each mouse received an injection of either vehicle (saline) or LPS. Additionally, 30 min before training, the mice received either vehicle or etomidate (2, 6, or 10 mg/kg, i.p.). Twenty-four hours after the training session, learning and memory performance was assessed by measuring the percent of time spent freezing. The experimenters were blinded to the drug treatment groups. Results: Etomidate caused a concentration-dependent impairment of contextual fear memory as evidenced by a decrease in the freezing scores, $F(2,42) = 24$, $p < 0.0001$. LPS further impaired contextual fear memory in a supra-additive manner, as demonstrated by the markedly lower freezing scores of the LPS-treated groups at all three concentrations of etomidate, $F(1,42) = 42$ ($p = 0.025$). Discussion: Inflammation induced by LPS intensified the memory blocking properties of etomidate. Specifically, LPS and etomidate interact in a supra-additive manner to reduce freezing scores in a contextual fear conditioning assay. The results suggest that memory-blocking doses of general anesthetics might be reduced in patients who experience systemic inflammation.

22. LIMK1 REGULATES SYNAPTIC PLASTICITY AND MEMORY VIA INTERACTION WITH CREB.

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The LIM-Kinase family of proteins (LIMK) is known to play an important role in actin dynamics through its regulation of ADF/coffilin. A subtype of LIMK, LIMK1, is only expressed in the neuronal tissues with high levels in the mature synapse. Previous studies from this lab have shown that LIMK1 knockout mice exhibit abnormal spine morphology as well as altered hippocampal synaptic plasticity. A recent study has found that LIMK1 phosphorylates the cyclic AMP Response Element Binding Protien (CREB) in hippocampal neuroprogenitor cells. CREB activation via synaptic activity facilitates late long-term potentiation (L-LTP) and long-term memory formation. We propose that LIMK1 is able to phosphorylate CREB in response to a synaptic activity. We hypothesize that if LIMK1 activates CREB in mature neurons, then LIMK knockout mice will have depressed L-LTP and deficits in long-term memory. Our results show that LIMK1 and CREB co-localize in mature neurons of the hippocampus. This is the case in both hippocampal slices and hippocampal cultures. Additionally, LIMK1 knockout mice exhibit altered L-LTP with a variety of induction protocols. Activation of CREB by application of forskolin is not able to completely rescue this deficit. LIMK1 lacking mice alos show a decrease in forskolin-induced LTP. Fear conditioning experiments show that LIMK1 -/- animals have a specific deficit in contextual long-term memory while their short-term memory remains intact. Morris water maze experiments show that LIMK1 lacking animals are able to learn the task as well as their wild-type littermates and perform well in the short-term memory test. However, LIMK1 knockout mice do not perform as well as wild-type controls in long-term memory tests in the Morris water maze. These results indicate a specific role of LIMK1 long-term memory and synaptic plasticity through regulation of CREB. (This study was funded by the Canadian Institute for Health Research)



Cardiovascular

23. RGS4-LACZ EXPRESSION IS ASSOCIATED WITH SPONTANEOUSLY BEATING CARDIOMYOCYTES DURING SINOATRIAL NODE DEVELOPMENT

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Parasympathetic-mediated hyperpolarization of sinoatrial-node (SAN) myocytes results in a decreased heart rate. Regulator of G-protein signaling 4 (RGS4) is selectively expressed in SAN myocytes and has been shown to be an important regulator for parasympathetic signaling in those cells. Our study aims to determine the importance of RGS4 expression as a marker of SAN cells during cardiac development. We here characterize changes in RGS4 expression in the heart through development in the mouse and mouse embryonic stem cells (mES cells). We hypothesize that RGS4 is highly expressed in early autorhythmic cardiomyocytes derived from cardiac neural crest and mesenchyme progenitor and thus will be a useful marker of both immature and mature SAN myocytes. Using the ES cell culture system and the RGS4-LacZ-gene-trap genetic mouse model, we investigated the expression of RGS4 during mouse heart development as well as during the differentiation of mES cells. RESULTS: 1.) Fetal mouse hearts were isolated and stained for LacZ expression. High expression of RGS4 was observed selectively in the SAN area starting at E11.5, whereas earlier time points showed ubiquitous RGS4 expression. 2.) RGS4-LacZ mES cells were differentiated to cardiovascular lineage using a serum-free differentiation protocol. Cardiac monolayers consisting of cardiomyocytes, vascular smooth muscle cells, fibroblasts and endothelial cells were stained with LacZ. RGS4 expression was exclusively associated with cells located in contracting areas of the culture and strongly correlated with cardiac troponin-T staining. To ensure that RGS4 expression corresponded to SAN type cells, immunohistochemistry for HCN4 was performed in parallel with the LacZ stain. Together, this indicated that RGS4 expression is strongly associated with autorhythmic and/or beating cardiomyocytes during development. Recently, RGS4-GFP mES cells were derived from RGS4-GFP mice for additional studies with RGS4 expression in the SAN type cells. Further studies will be carried out to establish the nature of these cells and whether their isolation can be used to selectively induce specialized SAN-like tissues in vitro and in vivo. (We gratefully acknowledge the support for this project by the Canadian Institute for Health Research Canada Research Chairs Program, the Canada Foundation for Innovation, and the Heart and Stroke Foundation of Canada.)

24. EFFECT OF PH ON ELECTROSTATIC INTERATIONS AND MYOFILAMENT LATTICE SPACING

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Previous studies have concluded that spacing between myofilaments in cardiac muscle is determined primarily by a balance between compressive forces arising from osmotic gradients and VanderWaals interactions and repulsive electrostatic forces which can be altered by changes in pH. Since cardiac force development and myofilament spacing are strongly dependent on sarcomere length, we examined the effects of pH on interfilament spacing as a function of sarcomere length in intact and skinned rat cardiac trabeculae. As expected from electrostatic theory, increasing pH=8 expanded the interfilament lattice spacing at sarcomere lengths of both 2.0μm and 2.2μm. However, reducing pH to 6.0 had no effect on the interfilament spacing at these sarcomere lengths, contrary to the predicted electrostatic effects of increasing proton concentrations. These results suggest that electrostatic forces play a minor role in myofilament lattice spacing. Indeed, solutions of the Poisson-Boltzmann equations for charged cylindrical rods in a lattice arrangement revealed that the electrostatic contributions to the myofilament spacing are small relative to the typical osmotic forces and, more important cannot account for the lattice changes observed with pH alterations. Our findings establish that the pH-dependent changes in myofilament lattice spacing in cardiac sarcomeres are independent of altered electrostatic interactions and suggest that pH influences myofilament lattice spacing via modulation of structural sarcomeric proteins.

25. DHHC ISOFORM MEDIATED REGULATION OF RGS4 LOCALIZATION AND FUNCTION.

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The etiology of cardiac arrhythmias are often unclear. Several hormones and neurotransmitters control cardiac electrophysiologic function via G-protein-coupled receptors making them important subjects of study in arrhythmia research. The regulator of G-Protein Signaling4 (RGS4) is highly expressed in the sinoatrial node of the heart and plays major roles in the regulation of M2R-mediated heart rate control. RGS4 elicits its effects at the plasma membrane (PM) and palmitoylation at cysteine positions

C2 and C12 has been demonstrated to be required for RGS4 translocation to the PM. A library of 23 mammalian palmitoyl-acyl transferase (PAT) enzymes have been cloned and characterized [Fukata et al, 2006]. All isoforms share a conserved Asp-His-His-Cys (DHHC) motif containing the catalytic site for PAT activity. We set out to establish the DHHC isoforms that regulate the localization and function of RGS4 in HEK cells. DHHC3 and 7 are localized to the Golgi in mammalian cells [Fukata et al, 2008] and have been shown to regulate trafficking and function of G-protein alpha subunits. Notably, knocking down DHHC3 and 7 with siRNA also decreased RGS4WT localization to the PM and its ability to inhibit the Gq-dependant calcium release in HEK cells. Moreover, functional palmitoylation of RGS4 by DHHC3 and 7 appears to occur wihtin the Golgi since overexpression of these DHHCs results in an increase in RGS4 accumulation in the Golgi compartment as demonstrated by its co-localization with TGN38. Lastly, mutation of C2 appears to have a much greater effect on DHHC targeting of RGS4 to the Golgi than mutating C12 suggesting that DHHC 3 and 7 may use C2 as their specific palmitoylation substrate.

26. VALIDATION AND MECHANISMS OF NOVEL COMPOUNDS MODULATING ANGIOGENESIS.

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Abnormal angiogenesis is featured in over 70 health conditions and is a critical component of tumour growth and metastasis. While inhibition of angiogenesis is an excellent target for cancer therapy, the stimulation of angiogenesis is beneficial to wound healing and tissue/organ regeneration. The zebrafish has emerged as an important model for studying vascular development and angiogenesis. Advantages over other models include rapid embryonic development and optical clarity of the embryos. The small body size of the zebrafish embryo and the external embryonic development within water make zebrafish embryos suitable for high throughput screen in 96 well plates.
In our previous study, we performed angiogenesis chemical genetic screens in two compound libraries (LOPAC and Spectrum Collection) using Flk1:EGFP (endothelial cells labeled green) transgenic zebrafish embryos. The compound bioactivity is measured by the numbers of the zebrafish trunk intersegmental vessels (ISVs). This study identified 10 antiangiogenic and three proangiogenic compounds. The goal of the current study is to validate the potential therapeutic efficacy of selected compounds and investigate their molecular mechanisms.
Indirubin-3-oxime (IO) and dihydromunduletone (DHM) were identified as antiangiogenic compounds. IO at 16 μM almost completely inhibited ISV development (ISV = 0.69 ±1.44, $p<0.05$) while the vehicle treated control embryos developed normally (ISV = 27.58 ± 0.76). DHM at 3 μM demonstrated some inhibitory effects ISV = 21.92 ± 9.38 ISVs ($p>0.05$). The effect of these compounds on physiological angiogenesis is being tested on larvae and adult zebrafish fin regeneration models. In larvae, 40 μM IO almost completely inhibits fin regeneration ($p<0.05$) and 2 μM DHM inhibits ~50% of fin regeneration ($p<0.05$). The efficacy of the compounds will be further studied in wound healing and cancer metastasis models in zebrafish and/or mice. Cellular and molecular mechanisms underlying the effects will be studied in human umbilical endothelial cells (HUVECs).

27. APELIN DEFICIENCY CONTRIBUTES TO CARDIAC FIBROSIS ASSOCIATED WITH ENHANCED VIMENTIN LEVELS

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Objectives: Apelin, the endogenous ligand for the G-protein-coupled APJ receptor, has been suggested to be involved in a broad range of physiological functions, including cardiovascular function, heart development and obesity. We aimed to determine how the lack of apelin activity affects cardiac performance under conditions of cardiovascular stress by working with apelin knockout (KO) mice.
Methods: C57BL/6 Apelin KO mice were previously generated and were subjected to either experimental myocardial infarction (MI) by left coronary artery ligation or pressure overload by aortic banding (AB). These procedures were done alongside their wild-type (WT) littermates. The mice were randomized for sacrifice post MI or AB for evaluation and comparison of function, morphology and molecular expression analysis
Results: The hearts of apelin KO mice exhibited more severe fibrosis in both the MI and AB models compared with WT littermates. Fibrosis-related genes were also up-regulated in the KO mice compared with the WT ones. Vimentin, an intermediate filament protein that is especially found in the interstitial space of the heart, was particularly increased post MI in the KO mice compared with the WT mice. It has been suggested that vimentin up-regulation indicates progressive development of fibrosis.
Conclusions: Apelin null animals compared with wild-type mice suffered from more severe fibrosis of the heart in both the MI and AB model, which could partially be explained by the significant enhancement in vimentin expression. Whether apelin is a direct upstream regulator of vimentin activity is yet to be confirmed. This is the first study to show that apelin is involved in regulation of a structural protein in the heart

28. THE ROLE OF TUMOR NECROSIS FACTOR ALPHA IN MICROVASCULAR TONE IN A MOUSE MODEL OF TYPE 2 DIABETES MELLITUS

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Diabetes is associated with structural and functional changes to resistance arteries that impair their ability to adequately autoregulate tissue perfusion and blood pressure. In this regard, we demonstrated that the myogenic response (MR), the intrinsic mechanism underlying the ability of resistance arteries to autoregulate, is enhanced in vessels from diabetic mice. Our previous work has identified microvascular sphingosine-1-phosphate (S1P2) signalling as the primary regulator of the MR in several vascular beds and demonstrated that the cytokine tumor necrosis factor α (TNFα) can affect the MR via modulation of S1P signalling. As both high-glucose treatment and diabetes upregulate vascular smooth muscle cell TNFα, we hypothesized that a TNFα-driven enhancement of microvascular S1P signalling underlies the diabetes-associated changes in myogenic responsiveness. MRs were assessed in mouse mesenteric arteries after induction of diabetes by high fat diet (HFD)/ streptozotocin protocol. Glucose levels were elevated in HFD/STZ mice. Passive diameter was significantly increased in vessels from diabetic mice. MRs were significantly augmented in diabetic mice compared to control (HFD-only) mice, while responses to phenylephrine were similar in both groups. Incubation with either the TNFα scavenger etanercept (1 mg/kg, in vitro) or the S1P2 receptor antagonist JTE013 (1 μmol/L) abolished increased MRs in diabetic mice. Under both conditions, responses to phenylephrine were fully maintained. Our data suggests that TNFα regulates MR changes in diabetes through the activation of microvascular S1P signalling, thereby identifying both pathways as potential therapeutic targets to mitigate the effects of microvascular dysfunction in diabetes. (This study was funded by an OGSST from the Heart & Stroke Foundation of Ontario (MS) and the Canadian Institute for Health Research)

29. ROLE OF TNF-ALPHA IN PAROXYSMAL ATRIAL FIBRILLATION.

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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. Ectopic beats arising from pulmonary vein (PV) cardiomyocytes play a prominent role in the development of paroxysmal AF, particularly in remodeled and diseased atria. Other factors such as reduction in action potential duration (APD), fibrosis and abnormalities in calcium handling have also been shown to play a role in development of this type of AF. The incidence of AF also correlates with inflammatory cytokines such as tumor necrosis factor-α (TNF-α), C-reactive protein and IL-6. TNF-α increases PV arrhythmogenic activity via abnormal calcium homeostasis. Mice with chronic overexpression of TNF-αshow increased susceptibility to AF, however the acute effects of TNF-α on AF susceptibility are not known. Hence, we hypothesize that TNF-α alters calcium homeostasis in atrial myocytes via an increase in ryanodine mediated spontaneous Ca2+ release (Ca2+ sparks) leading to delayed after depolarizations (DADs), premature atrial beats and triggered arrhythmias. Results: We have shown that short exposure to TNF-α increases the susceptibility to AF. Spontaneous calcium activity was measured in electrically paced isolated mouse atrial cardiomyocytes loaded with Fluo-3-AM dye and treated with 20ng/ml TNF-alpha for one hour, using confocal microscopy. Isolated atrial myocytes treated with TNF-α showed increased frequency of calcium sparks compared to control atrial myocytes (Spark *S -1 *(100μm) -1 = 5.46± 0.6 versus 2.67 ± 0.3 for controls), with increased incidence of delayed after depolarizations. These results suggest that TNF-α increases susceptibility to AF most likely through alteration of calcium handling and increased spontaneous calcium release. My future studies will explore whether atrial stretch induces AF via local shedding and production of TNF-α.

30. STUDY ON CELL DEFECTS DURING ATHEROGENESIS

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Migration and proliferation are important events for both atherosclerosis and cancer. Directed migration of arterial smooth muscle cells (SMCs) from the media to the intima is a key process in atherosclerotic plaque formation.
The role of cellular proliferation to atherosclerotic plaque development has been studied extensively; however, less studied is the fidelity of cell division.
In a previous study, we showed that RHAMM and ARPC5, two cytoskeletal associated proteins, are involved in rear polarization of the microtubule organizing center (MTOC) in rat artery neointimal SMCs during migration in interphase. In this study, we determined whether RHAMM is also required for cell division events such as spindle formation, chromosome attachment and segregation, and cytokinesis. Cultured neointimal SMCs from rat arteries treated with siRNA for RHAMM or with the PKC inhibitor bisindolylmaleimide for 6 hours after wounding had an increased number of multipolar spindles (tri- and tetra-polar

spindles) and centrosome defects controls. These inhibitory treatments also increased the frequency of binucleate cells compared to control neointimal SMCs. An actin net forms dorsolateral of the nucleus in control neointimal SMC. This net was displaced to the cell periphery or extended beyond the nuclear region in binucleate cells after RHAMM or PKC inhibition.
We are in the process of identifying interacting partners of RHAMM which may be involved in the control of centrosome polarity and spindle assembly.
In conclusion in SMCs, RHAMM controls centrosomal dependent processes both in interphase (rear polarization of the MTOC) and during division (spindle assembly, chromosome division and cytokinesis). The centrosomal targeting sequence of RHAMM is required for RHAMM function in the above processes. Our in vivo studies show that neointimal SMCs from injured rat carotid arteries and underlying medial SMCs exhibit elevated rates of proliferation, and the neointimal cell exhibit spindle and cell division defects.
L. Silverman-Gavrila was supported by a Fellowship from the Heart and Stroke Foundation of Canada, M. Bendeck was a Career Investigator of the Heart and Stroke Foundation of Ontario. This work was funded by operating grants to M. Bendeck (Heart and Stroke Fundation of Ontario T6084 and T6734) and to M. Charlton (CIHR MOP-82827). The poster is dedicated in the memory of Dr. Langille.

31. PDE3A MAY REGULATE MYOCARDIAL CA2+ CYCLING THROUGH INTERACTINGWITH SERCA2-SIGNALLING COMPLEXES IN MOUSE HEART

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cAMP is an important regulator of myocardial Ca2+ transients and function. By catalyzing hydrolysis of cAMP, cyclic nucleotide phosphodiesterases (PDEs) regulate the amplitude, duration, and compartmentation of cAMP–mediated signaling. The role of specific PDE isoforms in regulating cAMP-signaling pathways and cardiac functions has not been completely defined. For example, it remains controversial whether the PDE3A or the PDE3B gene is the primary PDE3 isozyme responsible for regulating basal Ca2+ transients and myocardial contractility. Thus, we used PDE3A-null mice to explore the role of PDE3A in regulation of baseline cardiac function and cAMP-mediated Ca2+ uptake into the sarcoplasimic reticulum (SR). Compared to littermate controls, heart homogenates from PDE3A-/- mice showed a 2-fold reduction in total PDE activity in conjunction with a nearly 10-fold reduction in PDE3 activity without changes in PDE4 activity, the other major PDE in the heart. Isolated PDE3A-/- hearts also showed elevations in contractility and relaxation properties while in isolated PDE3A-/- myocytes had elevated Ca2+ transient amplitudes and SR Ca2+ content, without differences in L-type Ca2+ currents (ICa,L), compared to WT. PDE3A-/- myocardium also showed increased Ca2+ uptake rates in SR vesicles and SR Ca2+ content in isolated myocytes. Ca2+ transient and SR Ca2+ content were normalized to the WT levels by dialysis of myocytes with the cAMP inhibitor RpcAMP. Although PDE3 inhibition had no effect on cardiac contractility, Ca2+ transients or SR Ca2+ content in PDE3A-/- preparations, it increased these same parameters in WT hearts to levels indistinguishable from PDE3A-/-. The functional changes observed in PDE3A-/- hearts were associated with enhanced phoshpo-phospholamban levels. In addition, we found that PDE3A co-immunoprecipitates with both SERCA2 and phospholamban. Our data demonstrate that PDE3A is the primary PDE3 isozyme modulating basal heart function and SR Ca2+ content by regulating cAMP in microdomains containing SERCA2-PLN-PDE3A macromolecular complexes. (This study was funded by the Canadian Institute for Health Research-PHB, National Institutes for Health-VM, and HCRLE postdoctoral fellowship-SB)

32. CLC-3 IS REQUIRED FOR SERUM-STIMULATED, BUT NOT HYPOXIA-INDUCED, ROLIFERATION IN HUMAN PULMONARY ARTERY SMOOTH MUSCLE CELLS.

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Growth factor-stimulated and hypoxia-induced proliferations of pulmonary artery smooth muscle cell (PASMC) lead to adverse vascular remodeling and contribute to pulmonary hypertension, a fatal disease with a 15% annual mortality rate despite current therapies. Our previous study demonstrated the expression of Cl- currents (ICl) in proliferating PASMCs and suggested a role of ICl in regulation of proliferation stimulated by serum (containing growth factors). However, it remains unknown which Cl-channel genes are expressed in PASMCs and their roles in serum- and hypoxia-induced proliferations. CLC family chloride channel genes play important roles in physiology and disease and CLC-3 has been suggested to encode swelling-activated ICl and play a role in proliferation in several cell types. Therefore, the present study first examined the expression of CLC genes in human PASMCs (hPASMCs). Quantitative real-time PCR demonstrated (n=4) the expression of 6 CLC family genes in proliferating hPASMCs, with the relative mRNA abundance being CLC-3(17.5) > CLC-7(5.3) > CLC-5(2.8) ≈ CLC-6(2.6) > CLC-4(2.0) > CLC-2(1.0). Since CLC-3 is highly expressed,

we examined effects of CLC-3 inhibition on serum- and hypoxia-induced proliferations in hPASMCs. Stable expression of a microRNA-adapted shRNA targeting CLC-3 in hPASMCs via lentiviral transduction reduced ($p<0.01$) CLC-3 mRNA by 80% (22.0±1.3 vs 4.2±0.7 arbitrary unit, n=3) and did not reduce the expression of other CLC genes. Serum-stimulated cell number increase in hPASMCs expressing CLC3-shRNA was reduced ($p<0.01$) by 46% [cell numbers (×104 cells/well) at day 4 in culture were 3.7±0.3 in control-shRNA vs 2.0±0.2 in CLC-3-shRNA, n=4]. In contrast, hypoxia (1%O2 for 3 days)-induced cell number increase was not reduced by CLC3-shRNA expression in hPASMCs. In addition, CLC-3 mRNA level was not affected in hPASMCs after hypoxia (1%O2 for 3 days) exposure. These findings are consistent with the conclusion that CLC-3 is expressed in hPASMCs and play a role in serum-stimulated, but not hypoxia-induced proliferation. Effects of CLC-3 knockdown on ICl in hPASMCs are currently under investigation.(This study was funded by the Canadian Institutes for Health Research).



E.D.R.G.

33. INSULIN RESISTANCE EFFECTS HYPOTHALAMIC LEPTIN REGULATION IN PROOPIOMELANOCORTIN NEURONS

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The main hindrance to developing effective strategies to control energy homeostasis at the level of the central nervous system is the development of resistance to peripheral signals, such as insulin and leptin, as seen in obesity and type 2 diabetes mellitus (T2DM). Mounting evidence suggests that environmental factors known to trigger peripheral insulin resistance may also affect the hypothalamus directly. However, the precise molecular mechanisms underlying neuronal insulin and leptin resistance remain unclear, mainly due to the complexity of the in vivo architecture of the hypothalamus and the lack of appropriate cell models for these studies. To this end, our laboratory has generated an array of immortalized hypothalamic cell lines. Using RT-PCR, we have identified a rat, embryonic cell-line, rHypoE-19, that endogenously expresses POMC, the long-form leptin receptor (Ob-Rb), insulin receptor (IR) as well as key enzymes necessary for the processing of POMC into □-melanocyte-stimulating hormone, prohormone convertases (PC) 1/3 and 2. We hypothesize that insulin resistance at the level of individual hypothalamic neurons can directly hinder leptin sensitivity. Using real-time RT-PCR we have demonstrated that 10 nM leptin and/or 10 nM insulin treatment regulates PC1, SOCS3, IRS-1, IR, and POMC transcript levels at 8 and 24 hours following treatment. Furthermore, we have established that insulin regulates these neurons through the PI3K-Akt and MAPK signaling pathways, as determined by phosphorylation of Akt (PKB) and extracellular signal-regulated kinase (ERK), respectively, upon IR activation over a 60 minute time course. We have also developed a model of neuronal insulin resistance via prolonged insulin exposure. Exposure of neurons to 100 nM insulin over 24 hours prior to insulin challenge demonstrated attenuation in insulin signaling as determined by a reduction in phosphorylated Akt. Currently we are investigating whether leptin can successfully activate leptin signalling in the context of insulin resistance by analyzing the effects of leptin on gene expression upon induction of insulin resistance. We also aim to determine if leptin resistance does in fact attenuate insulin sensitivity using a similar experimental paradigm. These findings will corroborate

the idea that environmental factors known to trigger insulin resistance may have consequences at the level of the individual hypothalamic neuron, which may ultimately contribute to the pathophysiological states of obesity and T2DM. (This research was supported by grants from the Canadian Institutes for Health Research (CIHR), Canada Foundation for Innovation (CFI) and the Canada Research Chairs (CRC) Program.)

34. MECHANISM UNDERLYING METFORMIN-INDUCED SECRETION OF GLUCAGON-LIKE PEPTIDE-1 FROM THE INTESTINAL L-CELL.

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EThe incretin hormone glucagon-like peptide-1 (GLP-1) is secreted by the intestinal L-cell in response to both nutrient and neural stimulation, leading to enhancement of glucose-dependent insulin secretion. GLP-1 is therefore a most attractive therapeutic approach for the treatment of Type 2 Diabetes Mellitus (T2DM). The anti-diabetic drug, metformin, has previously been shown to increase circulating levels of GLP-1, although its mechanism of action is currently unknown. To elucidate this mechanism, GLP-1 secretion was measured in murine GLUTag, human NCI-H716, and rat FRIC L-cell cultures treated with metformin (5, 15, 45, 150, 2000μM) or AICAR (100, 1000μM), activators of AMPK. Neither metformin nor AICAR directly stimulated GLP-1 secretion, despite a 1.7±0.2-fold increase in AMPK phosphorylation ($P<0.01$, $n=8$). Adult Wistar rats treated with metformin (300mg/kg, p.o.) and AICAR (250mg/kg, s.c.) showed an increase in plasma total GLP-1 over a 2h period, peaking at 37±9pg/ml and 29±9pg/ml ($P<0.001$) respectively, compared to basal (7±1pg/ml). Plasma activity of the GLP-1 degrading enzyme dipeptidyl peptidase-IV was not affected by metformin treatment, further indicating metformin's activity as a GLP-1 secretagogue. Pre-treatment with the non-specific muscarinic antagonist atropine (1mg/kg, i.v.) decreased the area-under-curve (AUC) of metformin-induced GLP-1 secretion by 55±11% ($P<0.05$). Pre-treatment with the M3 muscarinic receptor antagonist 4-DAMP (500μg/kg i.v.) also decreased the GLP-1 AUC by 48±8% ($P<0.05$), whereas the antagonists pirenzepine (M1) and gallamine (M2) had no effect ($n=6$-9). Furthermore, chronic bilateral subdiaphragmatic vagotomy decreased basal secretion compared to sham-operated animals (7±1pg/ml vs 13±1pg/ml, $P<0.001$, $n=8$), but did not alter the GLP-1 response to metformin treatment. In contrast, pre-treatment with the gastrin-releasing peptide (GRP) antagonist, RC-3095 (100μg/kg s.c.), reduced the GLP-1 response to metformin by 55±6% ($P<0.01$, $n=6$) at 30 minutes. Together, these studies emphasize the importance of the regulation of GLP-1 by this anti-diabetic drug, further enforcing the benefits of using metformin along with concurrent GLP-1 therapy in patients with T2DM.

35. THE ROLE OF TXNIP IN THE DEVELOPMENT OF DIABETIC NEPHROPATHY

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Thioredoxin-interacting protein (TxNIP) is an endogenous inhibitor of thioredoxin, a thiol oxidoreductase that regulates cellular redox status. TxNIP is upregulated by high glucose (HG) and has been shown to promote oxidative stress. We have found that Hcb19 mice, which lack TxNIP, are partially protected from streptozotocin-induced diabetes. Since HG and reactive oxygen species (ROS) are key mediators of the microvascular complications of diabetes, we investigated the potential role of TxNIP in the pathogenesis of diabetic nephropathy. Hcb19 and wildtype C3H mice were rendered equally diabetic with streptozotocin. While Hcb19 mice showed significant albuminuria in the non-diabetic state, in contrast to C3H, there was no increase caused by diabetes. While immunohistochemical analysis of glomeruli revealed no significant differences in TGFβ1 and collagen staining in Hcb19 and C3H controls, after 6 months of diabetes, glomerular accumulation of both were significantly increased only in C3H mice. To investigate the mechanism of protection, primary mouse mesangial cells (MC) were cultured from the 2 strains and exposed to HG. C3H MC exposed to HG (25 mM) for 3h showed a significant increase in intracellular ROS production (DCF, MitoSox), while Hcb19 MC had no response. HG-induced thioredoxin activity was decreased only in C3H MC ($p<0.001$). NADPH oxidase activity significantly increased only in C3H with HG treatment. Recently, the mitochondrial-localized NADPH oxidase isoform, Nox4 has been implicated in HG-mediated ROS generation. Nox4 protein was increased by HG only in C3H total cell lysates and isolated mitochondrial fractions ($p<0.05$). These data indicate that TxNIP is a critical component of the HG-ROS signaling pathway, apparently required for the induction of the mitochondrial NADPH oxidase isoform Nox4.
(This study was funded by the OGS, NSERC, and BBDC Graduate Studentships)

36. THE PROTECTIVE EFFECT OF PEROXIREDOXIN II OXIDATIVE STRESS INDUCED B-CELL APOPTOSIS IN THE PANCREATIC B-CELL LINE MIN6

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Oxidative stress plays a major role in the destruction of insulin producing β-cells and is a major contributor to the onset of both type 1 and type 2 diabetes mellitus. This is

primarily due to reduced
expression level of conventional antioxidants such as superoxide dismutase, catalases and glutathione peroxidises in β-cells. Peroxiredoxins are a group of ubiquitously expressed thioredoxin dependent peroxide reductases. All peroxiredoxins have been found to be expressed in pancreatic β-cells. I hypothesize that peroxiredoxin II (PRDX2) is an important factor in the protection of β-cells from
the effects of oxidative stress and subsequent apoptosis. To determine the role that PRDX2 plays in β-cells during oxidative stress induced apoptosis, PRDX2 is overexpressed via transient transfection or knocked down via siRNA in the pancreatic β-cell line Min6. The cells are then treated with oxidative stress agents such as proinflammatory cytokines, non-esterfied fatty acid (NEFA) and streptozotocin (STZ). Western blot analysis of the protein harvested from the treated cells revealed that cells overexpression of PRDX2 decreased cleaved caspase 3 expression, a well known apoptotic marker, compared with treated cells overexpressing GFP only. Consistently, nuclear stains of treated cells also showed that overexpression of PRDX2 has fewer cells with nuclear condensation and fragmentation, an indicator of the process of apoptosis, compared with treated β-cells overexpressing GFP only. These findings lead me to believe that PRDX2 does indeed play a protective role in pancreatic β-cells under oxidative stress and is an important component in the prevention of β-cell apoptosis.

37. LOSS OF LOCAL VASCULAR ENDOTHELIAL GROWTH FACTOR-A ACCELERATES DIABETIC NEPHROPATHY.

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Vascular endothelial growth factor-A (VEGF) is required for endothelial cell differentiation and survival. VEGF expression is dysregulated in patients with diabetes (DM) and nephropathy. Deletion or over-expression of VEGF in kidney podocytes leads to glomerular disease in mice. The administration of VEGF blocking agents to diabetic rodents has led to confusing and contradictory results. We hypothesized that the loss of local VEGF from podocytes of diabetic adult mice will accelerate the course of diabetic nephropathy, given the critical role of VEGF in normal glomerular biology. To investigate the renoprotective properties of VEGF in diabetes an inducible Cre-loxP gene targeting system was used to excise VEGF from podocytes of adult mice (VEGFKO). Results: Diabetes was induced by streptozotocin (STZ) at 2.5 weeks of age and VEGFKO was induced by doxycycline (Dox) at 3-4 weeks of age. Blood and urine were collected weekly to monitor for hyperglycaemia and proteinuria, respectively. Mice were dissected 8 weeks after diabetes induction or earlier if morbidly ill; twenty percent of the mice in the DM+VEGFKO group died before the surrogate endpoint. Glomerular VEGF mRNA expression was increased in diabetic mice compared to controls. However, DM+VEGFKO mice had significantly greater proteinuria, degrees of glomerular sclerosis, and glomerular cell apoptosis. These results confirm that VEGF is normally upregulated in diabetes but reducing VEGF expression in diabetes causes severe kidney injury. (This study was funded by the Research Assistant Stipend - National Institutes of Health (US) Grant, Monelle Siegal/Gozlau & The Samuel Lunenfeld Research Institute Fellowship (OSOTF), and the Banting and Best Diabetes Centre Graduate Studentship)

38. HIGH GLUCOSE-INDUCED ROS PRODUCTION IS MEDIATED BY C-SRC IN MESANGIAL CELLS.

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The pathogenesis of diabetic nephropathy (DN), a leading cause of end stage renal disease, remains incompletely understood. The pathologic changes caused by chronic exposure to high glucose (HG) require ROS. Mitochondria and NADPH oxidase(s) appear to contribute to the generation of ROS in response to HG. In previous studies, we observed the activation of the Tyr kinase Src by HG and showed that in HG Src is required for EGFR transactivation, stimulation of MAPKs and synthesis of collagen IV in cultured rat mesangial cells (MC). Src has been reported to be both upstream and downstream of ROS. To determine its role in DN, MC were exposed to either normal glucose (NG 5mM) or HG (25 mM) and ROS measured using DCF fluorescence in the presence and absence of Src family kinase inhibitors, Dasatinib and AZD0530, as well as after transfection with Src-specific siRNA. After 3 h HG significantly induced ROS production that was markedly decreased by Dasatinib, AZD0530, and Src siRNA. Src has been reported to phosphorylate Vav2, a GEF (guanine nucleotide exchange factor) for Rac1, which is involved in NADPH oxidase activation. HG stimulated Vav2 Tyr172 phosphorylation and Rac1 membrane localization, both of which were blocked by AZD0530. In addition, Src siRNA blocked HG-induced Vav2 phosphorylation. HG also stimulated the physical association of Src with Vav2 and Vav2 with Rac1 determined by co-immunoprecipitation. AZD0530 blocked Vav2-Rac1 association, indicating that the kinase activity of Src is required for Vav2-Rac1 signaling. In addition, Lucigenin-based NADPH oxidase activity was stimulated by HG and was abrogated by AZD0530. These data indicate that HG stimulates Src which subsequently, via Vav2-Rac1 NADPH oxidase and

the generation of a major proportion of ROS in MC. In light of our previous findings, Src-mediated ROS appears to be a major contributor to the pathologic changes seen in DN.

39. DETERMINING THE EFFECT OF MOLECULAR OXYGEN TENSION ON ISLET METABOLISM AND ENDOTHELIAL CELL MORPHOLOGY.

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Donor islets are injected into the portal vein of a recipient to treat type 1 diabetes. The donor tissue eventually becomes lodged in the presinusoidal capsule of the liver. Hypoxia and limited nutrients at this site result in endothelial cell (EC) death and beta-cell dysfunction until the islets can become revascularized with the recipient. Studies in other tissues have shown that hypoxia preconditioning and flow in vitro enhances cell survival post transplantation. To test the combined effect of preconditioning and flow on pancreatic islets, we exposed 10-12 week old C57BL/6 mouse islets to a hypoxia mimetic (100 μM CoCl2) and flow in a microfluidic device. This microfluidic device also allows subsequent measurement of the tissue using immunofluorescence labeling (PECAM-1), live cell fluorescence imaging (NAD(P)H, Ca2+), and effluent analysis (VEGF, insulin). EC connectivity measured using PECAM-1 immunofluorescence increased significantly (1.79- and 2.87-fold) in flow and CoCl2 + flow, respectively. VEGF secretion increased in islets treated with CoCl2 alone and CoCl2 + flow, but decreased in islets treated with flow alone. The NAD(P)H response in beta-cell mitochondria and cytoplasm was unaffected by flow or hypoxia; however, we observed a small 1.28-fold ($p<0.02$) increase in cytoplasmic NAD(P)H of islets in CoCl2 + flow. Overall, our results show that a combination of CoCl2 and flow enhances EC area and connectivity due to increased endogenous VEGF secretion. Our data suggest that CoCl2 and CoCl2 + flow increases glucose metabolism in the cytoplasm, which agrees with the shift in metabolism reported in the literature. Altogether, our data show that preconditioning improves EC morphology and increases glycolytic metabolism. Further studies will determine the effect on beta-cell insulin secretion.

40. THE ROLE OF SIRT1 IN PANCREATIC BETA CELLS

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Sirt1, a sirtuin family member, functions as a nicotinamide adenine dinucleotide (NAD)-dependent deacetylase. Importantly to us is its protective role in type II diabetes. It has been shown in mice that Sirt1 over-expression in pancreatic beta cells of BESTO mice caused increased insulin secretion upon challenge with glucose. Furthermore, whole body Sirt1 KO mice were previously shown to have a significantly dampened insulin response to glucose. However, studies thus far have been performed on whole-body knockouts of Sirt1 and so we have created and validated via means of PCR and qPCR, pancreatic beta cell-specific Sirt1 knockout (Sirt1 BKO) mice to study the role of Sirt1 in mediating aspects of type 2 diabetes. Oral glucose tolerance tests have shown that Sirt1 BKO mice exhibit a dampened ability to clear glucose from their blood and similarly, we measured insulin levels at the same time and found a markedly decreased level of plasma insulin. We have monitored the Sirt1 BKO mice's food intake, body weight however there is no significant difference compared to littermate controls. We further isolated Sirt1 BKO islets and performed in vitro studies such as glucose-stimulated insulin secretion to which we found these islets secret less insulin compared to controls. We further investigated islet morphology of Sirt1 BKO and found no apparent difference compared to control islets. And finally we began to investigate downstream targets on Sirt1 BKO mice in order to gain mechanistic insight into their aberrant phenotypes.

41. THE ANORECTIC CYTOKINE CNTF ACTIVATES HYPOTHALAMIC GHRELIN- AND UROCORTIN-EXPRESSING NEURONS BOTH IN VITRO AND IN VIVO.

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Ciliary neurotrophic factor (CNTF), a potent cytokine and neuronal growth factor, is anorexigenic in nature. We have demonstrated a role for CNTF in the immortalization of adult-derived neuronal cultures from mouse hypothalamus. However, the exact central mechanisms by which CNTF affects energy homeostasis or cell proliferation, and the extent to which CNTF influences other hypothalamic networks, has yet to be determined. To address this, we employed a clonal, immortalized mouse hypothalamic cell line generated in our lab. The mHypoE-20/2 line endogenously expresses the CNTF receptor and specific neuropeptides, such as orexigenic ghrelin and anorexigenic urocortin. Using real-time RT-PCR we examined the effects of CNTF on the mRNA levels of ghrelin and urocortin in the mHypoE-20/2 line. We found that treatment of 10 ng/mL CNTF significantly increased ghrelin mRNA by 0.7-fold at 4 h, suppressed ghrelin mRNA by 43% at 48 h, and suppressed urocortin mRNA by 56% at 48 h, compared to vehicle-treated controls. This suggests that ghrelin and urocortin may function as downstream mediators through direct CNTF receptor activation. Therefore, we set out to elucidate the effects of CNTF on ghrelin and urocortin neurons in vivo. We performed intracerebroventricular injections of 0.5 mg/mL CNTF into mice, and examined its effects on ghrelin and urocortin neurons 2 h post-exposure.

Through double-label immunohistochemistry using specific antibodies against c-Fos, a marker of neuronal activation, ghrelin, and urocortin-2, we showed that central CNTF administration significantly activated ghrelin neurons in the dorsal dorsomedial nucleus and periventricular area, urocortin-2 neurons in the ventromedial hypothalamus, and significantly activated both ghrelin and urocortin-2 neurons in the arcuate, ventral dorsomedial and paraventricular nuclei. In conjunction, both our in vitro and in vivo studies suggest a potential role for CNTF in regulating hypothalamic ghrelin- and urocortin-expressing neurons to mediate its recognized effects on energy homeostasis and neuronal proliferation. (This study was funded by Canadian Institutes for Health Research (CIHR), Canadian Diabetes Association (CDA), Canada Foundation for Innovation (CFI), Banting and Best Diabetes Centre (BBDC), the Canada Research Chairs (CRC) Program and the Ontario Graduate Scholarship (OGS) Program.)

42. ROLE OF FATTY ACID TRANSPORT PROTEINS IN OLEIC ACID-INDUCED SECRETION OF GLUCAGON-LIKE PEPTIDE-1.

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Glucagon-like peptide-1 (GLP-1) is an intestinal L-cell hormone, released in response to nutrient ingestion, which enhances glucose-dependent insulin secretion. The monounsaturated fatty acid, oleic acid (OA), is an effective GLP-1 secretagogue and its actions are mediated via the atypical protein kinase C isozyme PKCζ. However, the mechanism by which OA crosses the plasma membrane is unknown. Previous studies in the murine GLUTag L-cell model demonstrated expression of mRNA transcripts for fatty acid-transport proteins CD36 and FATP1, 3 and 4. We hypothesized that one of these proteins plays an essential role in OA-induced GLP-1 secretion. Immunoblotting demonstrated the presence of all 4 transport proteins in GLUTag cells. The cells also demonstrated specific 3H-OA uptake for up to 60 min, which was dose-dependently inhibited by 0.5 and 1.0 mM unlabeled-OA ($P<0.001$). Furthermore, different mechanisms appear to be involved in OA uptake by the L-cell, as indicated by an increase in the slope of the uptake curve at t=45 min (t=0-45 min: 0.6+0.1; t=45-60 min: 1.8+0.2; $P<0.001$). Phloretin (200 μM), a non-specific inhibitor of carrier-mediated transport, significantly decreased 3H-OA uptake at both early (t=5-15 min, $P<0.001$) and late time points (t=60 min, $P<0.05$). Two separate approaches to knockdown FATP4 by siRNA resulted in 20+3% and 27+6% knockdown, respectively, and decreased 3H-OA uptake at t=60 min ($P<0.05$). Treatment of GLUTag cells with 0.5 and 1.0 mM OA dose-dependently increased GLP-1 secretion by 24+9% and 59+9%, respectively ($P<0.05$-0.001), while phloretin ($P<0.01$) and FATP4 knockdown ($P<0.05$) decreased OA-induced GLP-1 secretion by up to 47+6%. OA injected directly into the ileum of WT mice increased plasma GLP-1 levels at 15 and 60 min; in contrast, preliminary findings suggest decreased GLP-1 levels in FATP4 null mice at 60 min. Collectively, these findings indicate an essential role for FATP4 in the regulation of OA-induced GLP-1 secretion.

43. GLUCAGON-LIKE PEPTIDE-2 DIRECTLY REGULATES HYPOTHALAMIC NEURONS EXPRESSING NEUROPEPTIDES LINKED TO APPETITE CONTROL

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Glucagon-like peptide-2 (GLP-2) is a peptide hormone derived from post-translational processing of preproglucagon in the intestinal L cells, caudal brainstem, and the hypothalamus. Although, the major biological action of GLP-2 is intestinotrophic, it also regulates energy homeostasis via effects on gastric motility, nutrient absorption, and maintenance of gut mucosal integrity. However, the biological actions of GLP-2 on hypothalamic appetite-regulating neuropeptides remain poorly understood. To gain better insight into the central effects of GLP-2, we used the recently generated adult-derived clonal, immortalized hypothalamic cell line, mHypoA-2/30, that endogenously expresses the GLP-2 receptor (GLP-2R) and appetite-regulating neuropeptides, such as ghrelin and neurotensin. We studied the activation of signaling cascades triggered by a degradation-resistant GLP-2 analog, human (Gly2) GLP-2 [h(Gly2)GLP-2], and the resulting changes in the mRNA levels of ghrelin and neurotensin. Treatment of mHypoA-2/30 cells with h(Gly2) GLP-2 (10 nM) stimulated phosphorylation of CREB/ATF1 and ERK1/2, with significant upregulation of ghrelin mRNA transcripts by 95% and neurotensin mRNA transcripts by 50% at 24 h post-treatment. To further assess whether hypothalamic neuropeptidergic neurons are activated by GLP-2 in vivo, we intracerebroventricularly injected h(Gly2) GLP-2 into mice and analyzed activation of hypothalamic ghrelin and neurotensin neurons at 2 h post-treatment. By double-label immunohistochemistry with c-Fos, a marker of neuronal activation, we demonstrated that in the hypothalamic arcuate, dorsomedial, paraventricular nuclei, and in the lateral hypothalamic region, central h(Gly2)GLP-2 robustly activated ghrelin- and neurotensin-expressing neurons, whereas in the ventromedial hypothalamic nucleus only neurotensin-expressing neurons were activated. Taken together, our findings from both in vitro and in vivo studies provide evidence for a previously unrecognized link between the GLP-2R activation and hypothalamic neuropeptides involved in appetite-regulation. Specifically, the findings establish that neurotensin and ghrelin are regulated by GLP-2 in the hypothalamus, and

this regulation may play a role in the GLP-2R-mediated control of appetite and energy homeostasis.

44. THE ACTIONS OF GLUCAGON-LIKE PEPTIDE-2 IN MURINE COLITIS-ASSOCIATED CANCER.

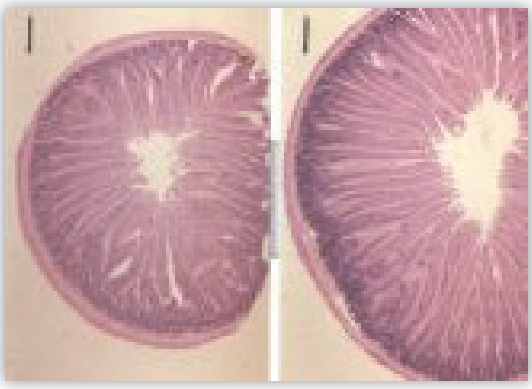
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Background: Glucagon-like peptide-2 (GLP-2) is a nutrient-induced intestinal growth factor with many beneficial actions, including increased crypt cell proliferation in the small and large intestine.. GLP-2 also ameliorates the severity of intestinal inflammation in rodents. Hence, a degradation-resistant GLP-2 analog is in clinical trials for treatment of inflammatory bowel disease (IBD). As colon cancer risk in increased in patients with IBD, we thereforeexamined the potential carcinogenic role of exogenous and endogenous GLP-2 in a cancer-prone IBD environment.
Methods: Adult, male C57BL/6 mice were injected with the colonic carcinogen AOM (10 mg/kg), followed by 1 wk of recovery and 3 cycles of the cytotoxic agent dextran sodium sulfate (DSS, 2.5%) (1wk) plus recovery (2wk) to simulate a murine model of cancer with chronic colitis. During the last recovery period, mice were injected (sc, bid) with vehicle, h(Gly2)GLP-2, or the GLP-2 receptor antagonist GLP-23-33 (n=26-35/group). Intestinal growth, damage and tumour analyses were performed.
Results: Mice treated with h(Gly2)GLP-2 demonstrated a significant increase in small intestinal weight by 54%, crypt depth by 13% and villus height by 50%. In contrast, antagonism of endogenous GLP-2 by treatment with hGLP-23-33 did not affect any of these parameters of small intestinal growth. Mice in all three groups had similar colonic damage scores, colon weights and lengths, although colonic proliferation was significantly increased by h(Gly2)GLP-2 treatment. While treatment with h(Gly2) GLP-2 did not increase carcinogenesis, blocking the actions of endogenous GLP-2 with hGLP-23-33 led to a 55% decrease in cancer incidence compared to vehicle-treated mice, as assessed by infiltration of tumour cells into the submucosa and/or muscularis. Conclusion: Exogenously-administered h(Gly2)GLP-2, does not alter cancer incidence in the murine AOM/DSS-model of colitis-associated cancer. Nonetheless, blocking the action of endogenous GLP-2 decreases cancer incidence. These data suggest a pro-oncogenic role of endogenous GLP-2 in colon cancer associated with chronic inflammation.

45. DIRECT SEROTONIN-MEDIATED SIGNALING AND DOWNSTREAM EFFECTS IN A HYPOTHALAMIC NEURONAL CELL MODEL

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Serotonin (5-HT) has been implicated in the control of feeding regulation. Infusion of 5-HT 1B receptor (5-HT1BR) agonists into the paraventricular nucleus of the hypothalamus (PVN) reduces food intake in mice. Moreover, 5-HT1BR-knockout mice have demonstrated that expression of the 1B receptor subtype in the PVN is required for the actions of fenfluramine, a potent appetite suppressant that elevates central serotonin levels. However, recent evidence suggests that 5-HT1BR activation may occur pre-synaptically on γ-aminobutyric acidergic inputs into the PVN. To investigate whether 5-HT acts directly on PVN neurons through the 5-HT1BR to elicit a downstream response, we have employed a novel PVN neuronal cell model. Within a vast phenotypic array of clonal hypothalamic cell lines generated in our lab, we have thoroughly characterized the expression profile of mHypoA-2/30 neurons and found them to represent a potential PVN neuronal model. These neurons express a complement of neuropeptides and receptors specifically localized to the PVN. Furthermore, the mHypoA-2/30 neurons express high levels of the 5-HT1BR, and minimal levels of 5-HT2AR, which is characteristic of the magnocellular PVN. We hypothesize that 5-HT has direct regulatory effects on PVN neurons, and that this effect is in part mediated by the 5-HT1BR. Preliminary findings demonstrate that 5-HT induces neuronal activation in a dose-dependent manner (100 nM-10 uM), as assessed by cFos mRNA levels. However, 5-HT does not significantly regulate the feeding-related peptides, nucleobindin-2, galanin, and neurotensin. Global secretome analysis to investigate the potential effect of 5-HT on neuropeptide secretion is currently underway. Finally, we are investigating the signal transduction pathways that underlie direct serotonergic regulation of these neurons. We expect that these novel PVN cell models can be used to delineate components involved in direct serotonin action in hypothalamic neurons. (We thank the Canadian Institute for Health Research (CIHR), Canada Research Chairs (CRC), Canada Foundation of Innovation (CFI), and Banting and Best Diabetes Centre (BBDC) for their funding support.)



Reproduction and Development

46. EXPRESSION OF PRO- AND ANTI-ANGIOGENIC ISOFORMS OF VEFA IN THE PLACENTA AND MATERNAL ORGANS DURING PREGNANCY IN MICE.

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Through alternative splicing VEGFA can act either as potent angiogenic or anti-angiogenic factors. Pro-angiogenic VEGFA120/164 increases in maternal plasma in late gestation but its source and function are unknown. The recently discovered and believed to be anti-angiogenic isoform, VEGFA165b, is expressed in the human placenta but its role in pregnancy is also poorly understood. Given the increase in vessel growth that occurs during pregnancy, we hypothesized that the expression of VEGFA120/164 would increase, and VEGFA165b would decrease, in the placenta and/or maternal organs during pregnancy. VEGFA120/164 and VEGFA165b proteins were measured in the placenta and maternal organs by ELISA in non-pregnant and pregnant ICR female mice. VEGFA120/164 protein in the ovary increased seven-fold by E13.5 and remained high at E17.5 consistent with a high level of ovarian angiogenesis in pregnancy. VEGFA120/164 did not change significantly in the kidney, lung, and heart. Contrastingly, VEGFA120/164 decreased in the decidua by 23-fold and in the placenta by 13-fold between E9.5 and E13.5 and remained low at E17.5 ($p<0.0001$). This is surprising because vascularity markedly increases in the placenta during this time. However, VEGFA165b in the placenta decreased approximately two-fold by E13.5 and remained low at E17.5 ($p=0.009$) potentially promoting vascularity. A two-fold decrease in VEGFA165b was also seen in the ovary, but expression in the kidney and lung did not significantly change with gestation. Results suggest that high levels of VEGFA120/164 in plasma in late gestation (E13.5 to E17.5) may be due to high expression of VEGFA120/164 in the ovary. Contrary to our expectations, VEGFA120/164 expression in the placenta and decidua decreased in late gestation so they are not likely to be the source. Decreased VEGFA165b in the ovary and placenta at E13.5 may promote angiogenesis but continued angiogenesis to E17.5 appears to involve other angiogenic mechanisms. This study was funded by the Canadian Institute for Health Research - MOP-93618.

47. CHARACTERIZATION OF BOVINE OVIDUCTAL EPITHELIAL CELLS IN CULTURE AND RESPONSE TO FOLLICULAR FLUID EXPOSURE (WORK IN PROGRESS).

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Recent theories on the pathogenesis of epithelial ovarian cancer suggest that high-grade serous type ovarian carcinomas originate from the distal fallopian tube epithelia. Studies suggest that ovulation is a micro-inflammatory event, of which a delayed resolution may lead to cellular transformation and precursor lesions within the fallopian tube epithelia. We are interested in studying the mechanisms involved in this resolution using an in vitro culture system. Due to the limited number of fallopian tube epithelial cells donated for research, bovine oviductal epithelial (BOE) cells serve as an alternate source of oviductal cells that can be obtained in large numbers and can serve to optimize culture conditions for adaptation to human culture and for use in mechanistic studies. The objective of this study is to characterize BOE cells in culture and to determine if exposure to human follicular fluid results in a pro-inflammatory signalling profile. BOE cells were mechanically isolated from oviductal tissue and seeded upon either transwell inserts coated with human placental type IV collagen, or collagen IV-coated wells. Cells were grown to confluence and immunocytochemistry was performed to examine expression of epithelial and mesenchymal cell markers. BOE cells grown on collagen-coated transwells exhibited a tight cobblestone-like orientation whereas cells grown on collagen-coated wells had an elongated fusiform appearance. BOE cells grown under either condition stained positive for oviductin, as well as cytokeratin epithelial cell markers cytokeratins, FoxJ1 (ciliated cells), and Pax8 (secretory cells). Staining for vimentin was observed in a small subset of cells. Human follicular fluid samples collected from IVF patients with informed consent were quantified for cytokine content. The human follicular fluid contained numerous inflammatory cytokines and growth factors. Following MTT assay, exposure of BOE cells to follicular fluid for 24 hours did not adversely affect BOE cell number or viability. Upon defining optimal culture conditions, experiments to test the impact of follicular fluid on gene expression are underway and will be determined by high throughput gene arrays. BOE cells grown in primary culture on collagen type IV

reflect the cell types present in the oviductal epithelium. (This study was funded by the Canadian Institute for Health Research and the University of Toronto Department of Physiology Fellowship Award)

48. GLI3R CONTROLS URETERIC BUD BRANCHING MORPHOGENESIS IN A MOUSE MODEL OF PALLISTER-HALL SYNDROME.

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Pallister-Hall syndrome (PHS), characterized by renal hypoplasia, renal agenesis and hydronephrosis is caused by a truncating mutation in GLI3 that constitutively mimics the transcriptionally repressive arm of the Hedgehog (HH) signaling pathway. While HH pathway activation and down-regulation of GLI3 repressor (GLI3R) is required for embryonic kidney induction and differentiation of renal medullary components, GLI3R is required in the ureteric bud (UB) tip-cells for nephrogenesis. To understand how obligate GLI3R contributes to renal hypoplasia in PHS, we used the Gli3Δ699 mouse model (Bose et al., 2002) to explore kidney development. Gli3Δ699/Δ699 (mutant) mice have severe hydronephrosis and renal hypoplasia or a duplex collecting system. At E15.5 HH signaling activity is indicated by robust PTC1lacZ staining in the periureteric mesenchyme, medullary stroma and medullary UB and is markedly reduced in mutant kidneys in the periureteric mesenchyme and medullary stroma and is undetected in the medullary UB. Mutant kidneys have a 57% incidence of renal hypoplasia while the remaining 43% have a duplex collecting system at E15.5. Renal hypoplasia is characterized by a 53% decrease in kidney volume ($p<0.0001$) with a 43% decrease in mature glomeruli ($p=0.0074$). IF for nephrogenic structures (CITED1, NCAM, WT1) reveal comparable proportions of structures in both mutant and WT kidneys suggesting that the nephrogenic process is unimpeded. Renal hypoplasia is preceded by a 51% decrease in UB tip number at E12.5 ($p<0.0001$). UB tip-cell gene expression of Ret and Wnt11 at E13.5, required for UB branching morphogenesis, is markedly reduced by in situ hybridization. Intriguingly, replacing one Gli3Δ699 allele with a Gli3null allele genetically reduces the amount of GLI3R in the absence of full-length GLI3 and restores kidney phenotype at E18.5. Taken together these data suggest that GLI3R inhibits branching morphogenesis by controlling UB gene expression in a dose-dependent manner and leads to renal hypoplasia in PHS.

49. ANTENATAL DIETARY RESTRICTION IMPAIRS FETAL METABOLISM AND IS IMPROVED BY A DIET ENRICHED IN OMEGA-3 FATTY ACIDS.

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Reduced maternal dietary intake during gestation is associated with offspring developing the metabolic syndrome (insulin resistance, glucose intolerance, and obesity). Subjecting C57BL/6J mice to antenatal dietary restriction (ADR) simulates this phenomenon, and late-gestation males display elevated blood glucose and expression of hepatic gluconeogenic enzymes. Their progression towards insulin resistance is not yet fully characterized. Dietary omega-3 (ω3) fatty acids prevent development of a diet-induced insulin resistance. Studying ADR offspring, we hypothesized that: 1) they begin developing hepatic insulin resistance by late gestation and, 2) increasing postnatal dietary ω3 fatty acids will inhibit males from developing metabolic syndrome symptoms. Methods: Pregnant mice were fed ad libitum (controls, C) or a 30% calorie-reduced diet (R) from gestational day (d) 6.5 to 17.5. In study 1, 6h after injecting mice intraperitoneally with 30 µCi [U-14C]D-glucose on d18.5, incorporation into fetal hepatic glycogen was measured. Fetal serum insulin levels were quantified in separate litters. In study 2, weaned males were fed the control diet (~1% ω3 fatty acids) or an enriched ω3 diet (~35% ω3 fatty acids). Glucose tolerance testing was conducted at 3 and 12 months of age. Body composition indices were measured at 12 months of age. Results: In study 1, R offspring compared to controls synthesized less glycogen and had similar serum insulin levels. In study 2, at both timepoints, R/ω3 mice had improved insulin resistance compared to R/C. At 3 months, C/ω3 had unchanged insulin resistance compared with C/C, and the ω3 diet improved glucose tolerance for R and C groups. R/ω3 had a lower percentage of body fat than R/C, but C/ω3 did not differ from C/C. These findings suggests that R offspring develop hepatic insulin resistance during gestation and the likelihood of fulfilling this fated development of the metabolic syndrome can be hindered by dietary ω3 fatty acids. (This study was funded by the Canadian Institute for Health Research)

50. PRO-INFLAMMATORY CYTOKINES INHIBIT MULTIDRUG RESISTANCE IN THE DEVELOPING BLOOD-BRAIN-BARRIER.

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Phosphoglycoprotein (P-gp) extrudes a wide range of endogenous and exogenous (chemotherapeutics) substrates from cells. P-gp expression in microvessels of the fetal guinea pig brain increases dramatically towards term and into the early postnatal period. This coincides with a decline in protection provided by placental P-gp.

During this period the developing brain is susceptible to drugs and teratogens. Pro-inflammatory cytokines, produced in response to infection, are potent inhibitors of P-gp function in the liver. It is currently unknown whether cytokines can affect P-gp function in brain endothelial cells (BECs), which form the primary blood-brain barrier (BBB), during development. Studies were initiated using cells derived from 14-day-old guinea pigs to establish a BEC model, in which to investigate regulation of P-gp function during fetal and neonatal development. We hypothesized that pro-inflammatory cytokines inhibit P-gp function in BECs. Primary BEC cultures were established from 14-day old guinea pigs. Confluent cultures were treated with varying doses (100-104 pg/mL) of interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor α (TNF-α) for 1, 4 or 24h. Cells were then incubated for 1h with calcein-AM (1μM; P-gp substrate). Accumulation of fluorescent calcein was measured to determine relative changes in P-gp function with each treatment. IL-1β significantly reduced P-gp function (increased cellular calcein accumulation) at 1, 4 and 24h in BECs ($P<0.01$). Treatment with IL-6 significantly decreased P-gp function at 4 and 24h ($P<0.05$), with no effect at 1h. TNF-α treatment resulted in a significant decrease in P-gp activity at 24h ($P<0.05$), with no effect at 1 and 4h. Calcein accumulation was increased by 36, 76 and 55% following treatment with IL-1β, IL-6 and TNF-α, respectively. Pro-inflammatory cytokines potently inhibit P-gp function in BECs. Given that fetal protection by the placenta decreases towards term, the fetal BBB becomes important for brain protection in late gestation. Our data suggest that in pregnancies complicated by maternal or intra-amniotic infection, the fetal BBB may be less effective in preventing drugs and teratogens present in the maternal and fetal circulations from entering the fetal brain.

51. SELECTIVE SEROTONIN REUPTAKE INHIBITORS (SSRIS) MODIFY DRUG RESISTANCE IN THE PLACENTA AND AT THE FETAL BLOOD-BRAIN BARRIER.

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P-glycoprotein (P-gp) is a member of the ATP-binding-cassette (ABC) superfamily. P-gp is expressed at high levels in the placental syncytiotrophoblast where it prevents xenobiotics present in the maternal circulation from entering the fetus. In cancer cells, P-gp is inhibited by selective serotonin reuptake inhibitors (SSRIs), resulting in increased intracellular accumulation of P-gp substrates. We have previously shown that SSRIs can modulate P-gp-mediated drug transport (P-gp activity) in brain endothelial cells. In this study, we hypothesized that the SSRI sertraline would decrease P-gp activity in the placenta and fetal blood-brain-barrier (BBB) thereby increasing drug transfer from mother to fetus and into the fetal brain. At embryonic day 15.5, pregnant mice were injected with either sertraline (10mg/kg or 1mg/kg) or vehicle. Each animal was simultaneously injected with [3H]digoxin (1μCi/30g; an effective marker of P-gp activity). Animals were euthanized 5, 60 or 240mins after injection to determine the time course of effect. Half of the fetuses in each litter were left intact with fetal membranes and amniotic fluid (to assess total transplacental drug transfer), and half were dissected (to determine drug transfer into the fetal brain). Maternal blood was also collected. Drug ratios were determined. At 5 and 60min, there were no differences in placental or fetal BBB drug transfer. However, at 240min, both doses of sertraline produced a significant decrease in fetal digoxin accumulation and a significant increase in fetal brain digoxin accumulation. This study presents the novel findings that sertraline (4h exposure) increases placental P-gp activity, resulting in decreased drug transfer to the fetus, at the same time as it decreases fetal BBB P-gp activity, resulting in increased drug transfer into the fetal brain. This suggests that P-gp regulation by the SSRIs is tissue-specific. These findings have important clinical implications with respect to fetal protection during maternal drug therapy in pregnancy. (This study was funded by the Canadian Institute for Health Research (FRN-84220) to SGM and WG and CIHR Graduate Studentships to MB and SP.)

52. CHARACTERIZATION OF ACID CERAMIDASE AND SPHINGOMYELINASE ENZYMES IN NORMAL AND PATHOLOGICAL HUMAN PLACENTA.

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Sphingolipids, classically thought to be purely inert structural elements of the cell membrane, have recently been recognized to act as bioactive lipid mediators in a variety of patho-physiological processes. In particular, ceramides (CERs) have been shown to be important second signal effector molecules regulating pro-apoptotic events. Preeclampsia (PE) is a pregnancy-related disorder associated with placental hypoxia/oxidative stress and exuberant trophoblast cell death. Sphingolipid metabolism, in particular CER expression, is regulated by the enzymes, acid ceramidase (AC) and sphingomyelinase (ASM) which are responsible for ceramide synthesis and breakdown respectively. The objective of this study was to characterize the expression and regulation of AC and ASM in the human placenta in normal and preeclamptic pregnancy. Protein expression levels of AC and ASM were assessed in normal and PE human placental tissue using Western Blot analysis. Human villous explants and choriocarcinoma

JEG3 cells were treated with sodium nitroprusside (SNP: 2.5 and 5 mM), a nitric oxide donor known to mimic oxidative stress. During early development, AC and ASM showed a unique pattern of expression, whereby AC protein and mRNA levels peak around 12 weeks and ASM protein levels increased with advancing gestation. Placental lysates from preeclamptic patients revealed a significant decrease in both mRNA and protein expression of AC as compared to age-matched controls (AMC). Conversely, ASM protein expression increased in PE relative to normotensive control placentae. Interestingly, increased ASM levels were identified as a double band which has been postulated to be either an ASM precursor protein or a glycosylation product. Exposure of JEG3 cells to SNP increased CER expression and this was accompanied by a decrease in AC and an increase in ASM levels. Altered expression levels of AC and ASM in preeclamptic placentae, induced by the oxidative stress status, are responsible for changes in the sphingolipid rheostat which it turn may contribute to the genesis of this disorder. (Supported by CIHR)

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

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Objective: 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. Methods: 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. Results: 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). Conclusions: 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.

54. THE ROLE OF DECIDUAL NEUTROPHILS IN MOUSE ANGIOGENESIS (WORK IN PROGRESS).

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During early pregnancy, a large infiltrate of immune cells is recruited to the decidua concurrent with extra-villous trophoblast invasion, implicating a significant role for decidual leukocytes in spiral artery transformation. Traditionally, neutrophils are not believed to participate significantly in spiral artery remodeling and decidual angiogenesis due to their near absence in the maternal decidua in the first trimester. We have recently identified a novel population of neutrophils residing in second trimester decidua, which infiltrate the decidual tissue and are found clustered around blood vessels. To eliminate the possibility that invasion of decidual neutrophils may simply be due to inflammatory processes associated with the termination of second trimester pregnancy, our aim is to determine whether a similar neutrophil population exists in the mouse. Using immunohistochemical analysis of intact formalin-fixed uterine horns of pregnant CD-1 mice euthanized at days (d) 6, 8, 10, 12, 14, and 18 of gestation stained with a neutrophil-specific marker (7/4), neutrophil number and localization was assessed by counting 7/4-positive cells within the mesometrial triangle. Neutrophils were localized in and around the developing maternal uterine arteries of the mesometrial triangle. At d6, neutrophils were observed clustered at the invading front of trophoblast and around maternal arteries. Preliminary quantification of staining shows the largest population of neutrophils in early gestation. Our results demonstrate that neutrophils can be found throughout gestation in the mouse, with neutrophil number highest during early gestation, the most active phase of angiogenesis. This may lend supporting evidence to our finding of neutrophils in

human decidua and a postulated angiogenic role. The pro- and anti-angiogenic properties of neutrophils in tumours has previously been shown by numerous groups – taken together with their close association with blood vessels, our data suggests that neutrophils perhaps play a more significant role in decidual angiogenesis and trophoblast invasion than previously thought. (This study was funded by the Canadian Institutes of Health Research (IHD-86232) and Physicians Services Inc.)

55. CHARACTERIZATION OF HYPOTHALAMIC GONADOTROPIN-INHIBITORY HORMONE CELL MODELS TO DEFINE THE HORMONAL REGULATION AND DIRECT SIGNALLING EFFECTS IN HYPOTHALMIC NEURONS .

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Reproduction is controlled by the hypothalamic-pituitary-gonadal (HPG) axis, which consists of a complex array of neuropeptides and hormones. The novel gonadotropin-inhibitory hormone (GnIH) system has emerged as a potent inhibitory regulator of neuroendocrine function, particularly within the HPG axis, though its distinct role is not well established in mammals. To date, there is a paucity of studies focusing on the regulation of hypothalamic GnIH, as well as its potential regulation of GnRH neurons, largely due to a lack of appropriate cell models. In order to address these studies, our lab has generated immortalized, clonal, murine cell lines derived from both embryonic and adult hypothalamic primary culture. We have identified a subset of cell lines that exhibit strong GnIH expression, as well as the melatonin receptor, the kisspeptin receptor, GPR54, and the estrogen receptors, ERα and ERβ, thus represent potential GnIH neurons. Similarly, we have characterized a subset of GnRH-expressing cell lines that co-express the GnIH receptor, GPR147, thus presenting the potential for a direct inhibitory mechanism of GnIH on the GnRH neuronal population. We hypothesize that (1) 17β-estradiol and kisspeptin are implicated in the direct regulation of GnIH expression and secretion and (2) that GnIH directly regulates hypothalamic neuropeptides like, GnRH by acting through its Gi protein-coupled receptor, GPR147 to induce signaling transduction pathways, ultimately leading to the inhibition of the HPG axis. Currently, studies are examining the direct and dose-dependent effects of 17β-estradiol and another RFamide peptide, kisspeptin on GnIH mRNA transcript levels. Furthermore, the GnIH- mediated regulation of GnRH gene expression is being examined to verify its direct inhibitory role in the mammalian HPG axis. We expect that these novel hypothalamic GnIH cell models can be used to further define the cellular mechanisms by which GnIH input and signal transduction mediates mammalian reproduction. Sources of Research Support: Canadian Institutes for Health Research (CIHR), Canada Foundation for Innovation (CFI), the Canada Research Chairs (CRC) Program and the Genesis Ontario Graduate Scholarship in Science and Technology (OGSST) Program.

56. NEGATIVE MODULATION OF TGF-SS SIGNALING IN OVARIAN CANCER CELLS: A ROLE FOR ANDROGEN AND VEPH1.

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Dysregulation of the tumour suppressor function of transforming growth factor-ß (TGF-ß) signaling is associated with the pathogenesis of ovarian cancer. Normal and malignant ovarian epithelial cells are growth inhibited by TGF-ß, however, selective loss of anti-proliferative response to TGF-ß may promote tumour growth. Meanwhile, numerous epidemiological studies have implicated androgens in the etiology or progression of epithelial ovarian cancer. Excessive androgen signalling may prevent TGF-β mediated growth inhibitory responses through transcriptional regulation of modulators of TGF-β signalling. Previous gene expression profiles generated from androgen-treated primary cultures of non-malignant ovarian surface epithelial cells and from malignant cells isolated from patients ascites revealed increased expression of ventricular zone expressed pleckstrin homology domain protein 1 (Veph1), a novel inhibitor of TGF-β signaling. We hypothesize that androgen inhibition of TGF-β signaling in ovarian cancer cells may involve androgen up-regulation of Veph1 expression. Human ovarian cancer cell lines, ES2 and SKOV3, were transiently transfected with wild-type androgen receptor (AR) cDNA to determine the effect of a potent androgen, 5α-dihydrotestosterone (DHT), on TGF-β signaling using reporter gene assays. Androgen treatment significantly reduced Smad-dependent TGF-β signaling in ES2 and SKOV3 cells transiently overexpressing AR as determined by reporter gene assays. For further studies, SKOV3 cells were stably transfected with wild-type AR (SK-AR) and unique clones were isolated. Expression of AR in SK-AR clones was verified by Western blot analysis, while reporter gene assays were used to examine AR activity. As expected, SK-AR clones stably expressing AR demonstrate significantly enhanced androgen signalling in the presence of DHT. To date, our results indicate that androgen inhibits TGF-β-induced transcriptional activity in ES2 and SKOV3 ovarian cancer cells. Ongoing studies will use SK-AR clones as models to determine the role of Veph1 in androgen function.

57. THE EFFECTS OF LACTOBACILLUS RHAMNOSUS GR-1 ON AMNION CYTOKINE AND CHEMOKINE PRODUCTION.

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Over 30% of all preterm births are driven by an infectious/inflammatory process and antibiotic treatment is largely ineffective. High incidences of preterm labour are associated with Bacterial Vaginosis, an alteration to the vaginal flora where endogenous lactobacilli decrease and pathogenic anaerobes increase. Ascending bacterial products and endotoxins induce an inflammatory cascade resulting in increased cytokine production from immune cells and intrauterine gestational tissues that activate pathways and ultimately lead to preterm labour. Lactobacillus rhamnosus GR-1 (GR-1) alters cytokine production in placental trophoblasts; however, the effects of GR-1 on amnion produced cytokines and chemokines are unknown. We hypothesize that GR-1 will promote increased anti-inflammatory cytokine, decreased pro-inflammatory cytokine and increased chemokine concentrations in lipopolysaccharide (LPS) and lipoteichoic acid (LTA)-treated cultured human amnion cells at term. Methods: Amnion was stripped and cultured from placentae received from women undergoing elective caesarean sections at Mount Sinai Hospital showing no signs of labour at term. Amnion cells were plated until confluent and subsequently starved and treated with GR-1 supernatant for 12 hours each. Cells were treated with LPS and LTA for 12 hours. Medium was collected and analyzed using ELISA and Bioplex. Results: Interleukin (IL)-6 increased in all groups (n=13, $p<0.05$) compared to control: GR-1 (1.82 fold), LTA (2.38 fold), GR-1+LTA (2.22 fold), LPS (2.47 fold), GR-1+LPS (2.61 fold). IL-8 was increased in all groups (n=13, $p<0.05$) compared to control: GR-1 (10.87 fold), LTA (8.32 fold), GR-1+LTA (13.18 fold), LPS (3.74 ng/ml), GR-1+LPS (9.84 fold). Tumor necrosis factor (TNF)-α was increased with LPS (368.34 pg/ml) and LTA (103.93 pg/ml) compared to control (below detectable limits, 4pg/ml) and was inhibited with GR-1: GR-1+LTA (19.90 pg/ml), GR-1+LPS (69.10 pg/ml). Conclusions: These findings suggest that GR-1 promotes chemotaxis while down-regulating pro-inflammatory cytokine production in the human amnion. (This study was funded by the Canadian Institute for Health Research - MOP 82799).

58. CHARACTERIZATION OF AN IMMORTALIZED GNRH-GFP NEURONAL CELL LINE FAC-SORTED FROM ADULT-DERIVED GNRH-GFP MOUSE PRIMARY CULTURE.

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Gonadotrophin releasing hormone (GnRH) has been widely studied for its involvement in the regulation of reproductive function. Yet, the underlying mechanisms triggering the direct cellular regulation of GnRH neurons are still not completely understood. GnRH cell lines currently available are clonal and have been isolated from hypothalamic tumors, thus it is difficult to judge their overall representation of GnRH neurons in vivo. Therefore, our lab has recently generated and immortalized an adult-derived GnRH cell line consisting of the entire population of GnRH neurons from GnRH-GFP mice, wherein primary hypothalamic cultures were isolated from 2-month-old mice. The primary cultures were treated with 10 ng/ml ciliary neurotrophic factor to induce neurogenesis and immortalized using simian virus (SV40) large T antigen with a neomycin resistance gene. Following immortalization and selection, cells were FAC-sorted based on GFP fluorescence with greater than 95% purity. These cells represent the entire population of mHypoA-GnRH/GFP neurons, and have not been further subcloned. Using specific antibodies, the neurons have been examined for GnRH and GFP protein using double label immunocytochemistry to confirm cell phenotype and purity. All neurons in culture co-expressed both GnRH and GFP. ELISA revealed that the cells undergo KCl-induced depolarization to enhance GnRH secretion. Subsequently, RT-PCR was utilized to confirm that the cells endogenously express neural markers. These cells also express Otx2, a putative marker of differentiated GnRH neurons. RT-PCR also confirmed the presence of estrogen receptor β (ER-β), GPR130, GPR54 (Kiss1R), GPR147 (GnIHR), and the active form of the leptin receptor (Ob-Rb). Whether this new line will be a more representative model of the entire adult GnRH neuronal population than the clonal cell lines available is yet to be determined. Studies are underway to determine hormonal regulation of these cells. Characterization of the mHypoA-GnRH/GFP cell line is ongoing and this information will be used to analyze the direct hormonal regulatory mechanisms on a model of the entire isolated population of GnRH neurons from the adult mouse hypothalamus. Sources of Research Support: Canadian Institutes for Health Research (CIHR), Canadian Diabetes Association (CDA), Canada Foundation for Innovation (CFI), the Canada Research Chairs (CRC). Nothing to Disclose: SAM, SSD, JAC, MLC, DDB

59. IDENTIFYING PATHWAYS INVOLVED IN OOCYTE FATE VIA REGULATION OF MCL-1.

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Various pathways governing oocyte survival have been published; however actual downstream determinants of oocyte fate have not yet been elucidated. The Scf ligand, expressed by granulosa cells, and its associated receptor Kit, expressed by oocytes, has been posited as the means of communication by which follicle fate is controlled. In fact, numerous studies have indicated an important role for the Scf-Kit interaction in the primordial-to-primary oocyte transition. Signals received via this communication can activate a number of downstream regulatory pathways that control oocyte fate. We believe Mcl-1 is a downstream regulator of oocyte survival, and have analyzed an Mcl-1 oocyte-specific conditional knockout mouse model to show the necessity of Mcl-1 in oocyte survival. Preliminary Data has shown that Mcl-1 oocyte-specific conditional knockout mice undergo Premature Ovarian Failure, with primordial oocyte loss as early as 3 weeks. This infers a role for Mcl-1 in oocyte survival. In this study, we investigate the regulatory that regulate Mcl-1 expression. To determine these pathways controlling Mcl-1, primary oocytes were collected from d8 wildtype animals and cultured in vitro, in the presence or absence of Scf. Treatments with Scf show a time-dependant increase in Mcl-1 protein levels via Western Blots. Treatment with LY294002, a PI3Kinase inhibitor, in d8 primary oocytes shows a decrease in Mcl-1 levels. Additionally, the GSK3 inhibitor IX, or LiCl, can cause an increase in Mcl-1 levels. Our data suggests a role for Mcl-1 as a pro-survival factor in the regulation of oocyte fate. We confirm the putative role of Scf in oocyte survival and show that regulation of oocyte survival via the PI3Kinase pathway does involve Mcl-1 stability.

60. LUNG TISSUE ENGINEERING: CHARACTERIZATION OF DECELLULARIZED LUNG SCAFFOLDS.

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Regenerative medicine has become one of the major frontiers in the treatment of diseased tissues, and its progress is in large due to the rapid recognition of the potential for stem cells as therapeutic options. The interaction of stem cells with the surrounding matrix environment is crucial for cell fate. Consequently the development of biomatrices that recapitulate the in vivo environment is key to driving the differentiation of stem cells into lung endoderm precursors and ultimately transplantable, fully functional, lung tissues. Recent publications have demonstrated the potential for decellularized natural tissue scaffolds as the ideal candidate for supporting growth and differentiation requirements of stem cells. However, no universal lung decellularization protocol is available to preserve a extracellular matrix (ECM) capable of sustaining stem cell growth and lung lineage-specific differentiation. The optimal decellularization protocol will effectively remove all cellular components while maintaining protein composition, biological activity, and mechanical integrity of the natural ECM. It is hypothesized that such scaffold will be capable of driving the unidirectional differentiation of human embryonic stem (hES) cells into distal lung epithelial cells. Rat cadaveric lungs were decellularized by sequential tracheal lavages and retrograde pulmonary arterial perfusion using a range of physical, chemical, and enzymatic treatments. The scaffolds were analysed for their structure and composition using tissue staining (Movat pentachrome stain, Hart's elastin stain, picro-sirius red collagen stain, DAPI) for ECM components (collagenI/IV, elastin, reticular fibers) and cellular remnants. This protocol was optimized to generate scaffolds with intact ECM composition. These scaffolds will now be tested for their potential to support endoderm-induced hES cell adherence and promote maturation into a lung epithelial phenotype (supported by CIHR).

61. REGULATION OF AUTOPHAGY BY NLRP5 IN PREIMPLANTATION EMBRYOS.

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Originally identified during a screen for autoimmune primary ovarian failure, NALP5 is an oocyte specific protein that plays a critical role during early embryo development. This is evident in Nlrp5-deficient females, where ovulation and fertilization are normal but developmental failure occurs shortly after. Despite this strong phenotype, very little is known about the function of NALP5 and the pathways affected by its deficiency. Autophagy has been recognized as a key event during oocyte to embryo transition, essential for the elimination of maternal products. Autophagy-protein-5 (ATG5) deficient embryos display early embryonic lethality - a phenotype very similar to Nlrp5-deficient embryos. We hypothesize that NALP5 plays an important role in the clearance of accumulated maternal products via regulation of autophagy. To investigate whether NALP5 deficient embryos exhibit decreased autophagy, we used immunocytochemistry to determine expression levels of cl-LC3, Lysotracker, Beclin-1 and the accumulation of Ub-proteins. Further, to establish whether mTOR signaling pathway contributes to the developmental arrest of NALP5 deficient embryos, we will determine mTOR phosphorylation status. We will also evaluate the mRNA expression of various mRNA targets from microarray analysis by qRT- PCR. Results: We have observed decreased lysosomal activity, evidenced

by decreased Lysotracker signal in Nlrp5-deficient embryos. Furthermore, decreased expression of cl-LC3 was also detected, consistent with improper activation of autophagosomes. Interestingly, this was accompanied by elevated expression of Beclin-1, which formed aggregates in the cytoplasm of Nlrp5-deficient embryos. Furthermore, the transcript microarray profile of Nlrp5-deficient oocytes revealed de-regulated expression of ATG10 and several de-ubiquitinating enzymes implicated in the regulation of protein degradation. These findings suggest that with limited autophagy, Nlrp5-deficient embryos likely fail to eliminate maternal products, degradation of which is mandatory for the proper transition from oocyte to embryo. These experiments will assess the impact of NALP5 on embryo survival and explore mechanisms that are responsible for maintaining normal preimplantation development. (This study is funded by the Canadian Institute for Health Research (CIHR) and Genesis Research Foundation.)

62. CHARACTERIZATION OF MYOMETRIAL CYTOKINE EXPRESSION AND LEUKOCYTE INFILTRATION DURING TERM AND PRETERM LABOUR.

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Previous studies in rat have shown increased expression of uterine cytokines prior to the onset of term labour in association with myometrial/decidual infiltration of peripheral leukocytes (PLs). We hypothesize that PLs are recruited to uterine tissues by locally produced cytokines where they contribute to the initiation of term and preterm labour (PTL). Methods: Pregnant CD-1 mice were euthanized on gestational day (d) 15, 17, 18.5, 19.75 (prior to labour), term labour (TL) and post partum. On d15 of gestation pregnant CD-1 mice were injected with RU486 (0.15mg, sc, n=15), LPS (0.125mg, intra-uterine, n=15) or vehicle (n=10/group). Animals were euthanized during PTL or 24 hours post injection/surgery. Immunolocalization of macrophages and neutrophils was defined using newCast stereology software and systematic randomized sampling. Protein analysis was performed using Bio-Plex Pro Mouse cytokine 23-plex assay (BioRad) Results: Specific antibodies were used to compare the level of macrophage (F4/80) and neutrophil (7/4) infiltration into the myometrium. Quantitative stereologic analysis suggests that there is a significant increase in macrophages prior to the onset of labour, while neutrophils increase post partum ($p<0.05$). Neither macrophages nor neutrophils infiltrated the myometrium during RU486-induced PTL, however neutrophils infiltrate the myometrium during LPS-induced PTL ($p<0.05$). These changes in leukocyte number were associated with significant changes in multiple cytokines in the myometrium during TL and PTL compared to d15 or corresponding vehicle controls ($p<0.05$). Our findings suggest that different mechanisms underlie LPS- and RU486-induced PTL with differential cytokine expression dictating leukocyte infiltration. These results support the role of immune cells not only in uterine activation leading to TL and PTL, but also in the process by which the uterus returns to its pre-pregnant state during the post partum period. This study was funded by March of Dimes.

63. MODEL OF AUTOPHAGY IN MURINE OOCYTES.

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Age-related reproductive decline and infertility are often linked to accelerated oocyte loss and ovulation of poor quality oocytes. We have previously found that aged oocytes have an increased mitochondrial DNA copy number, as well as increased lysosomal quantity. These findings have led us to hypothesize that defective autophagy might be involved in the pathology of aging. In order to explore the autophagic response in the oocytes, we have developed a new in vitro model that prevents the delivery of essential nutrients from the supporting cumulus cells to the oocyte. Specificaly, we used cumulus oocyte complexes (COCs) collected from female mice 44 hours after PMSG injection. The COCs were incubated in αMEM medium alone (Control), or in medium supplemented with 100 μM carbenoxolone (Cbx), a gap junction inhibitor. The oocytes were then stripped of the cumulus cells, and either further incubated or fixed for immunocytochemistry. The former group was incubated with the LysoTracker Red probe to visualize the lysosomal activity and with the MitoTracker Green probe to stain the mitochondria. The fixed oocytes will be used to assess the levels of key autophagic proteins, such as Beclin-1, LC3, and LAMP-2. Both live and fixed oocytes were imaged on a spinning disc confocal microscope and quantitated using Volocity software. We have found that the oocytes treated with Cbx had increased levels of LysoTracker Red staining, suggesting an increase in the number of mature lysosomes. They also displayed a reduced level of MitoTracker Green staining, indicating a reduction in the mitochondrial pool. Both these changes could be reversed with 3-methyladenine, an inhibitor of autophagy, and induced by treatment with rapamycin, an inhibitor of mTOR. Thus, we have successfully used gap junction inhibitors to induce oocyte starvation and have documented changes consistent with autophagy. Studying the process of autophagy and its dysregulation, which has been implicated in somatic aging and numerous diseases, can aide greatly in our understanding of reproductive aging. (This study is funded by the Canadian Institutes of Health Research.)

64. A NOVEL ROLE FOR PAR6 IN TROPHOBLAST FUSION.

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Human placental development is dependent upon the establishment of proper trophoblast cell differentiation events including fusion and invasion, shaping proper organogenesis. This study examines the contribution of polarity to trophoblast cell fusion, an area of research which remains elusive, by examining Par6 (Partitioning defective protein 6), a key regulator of cell polarity. Methods: Human placental tissues across gestation, preeclamptic placentae and age-matched controls were collected. To establish a role for Par6 in trophoblast fusion, primary isolated trophoblast cells were cultured at 3% and 20% oxygen and Par6 expression was examined spatially and temporally in conjunction with a polarity marker, Zona Occludin-1 (ZO-1). Par6 expression was assessed following forskolin treatment in BeWo cells. Par6 siRNA strategy was employed in BeWo cells and ZO-1expression was examined. Results: During early gestation Par6 expression exhibited a unique spatial and temporal pattern of expression as it switched its subcellular localization with advancing gestation. In primary trophoblast cells, Par6 levels were increased after 48 hours of exposure to 3% O2 and localized to tight junctions while at 20%, Par6 expression remained cytoplasmic. Following forskolin treatment, Par6 expression decreased which was associated with an increase in syncytin expression. Interestingly, Par6 expression was increased in preeclampsia relative to age-matched controls. Discussion: These findings provide novel insights into a role for Par6 in regulating trophoblast cell fusion via its effect on polarity during human placental development which may be disrupted and contribute to the pathogenesis of preeclampsia.
(This study was funded by the CIHR/IGH and OGS)

65. EFFECTS OF PRENATAL SYNTHETIC GLUCOCORTICOID TREATMENT ON LOCOMOTOR ACTIVITY AND ATTENTION.

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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 help reduce the risk of infant respiratory distress syndrome. In animal studies, prenatal exposure to sGCs has been associated with modification of hypothalamic-pituitary-adrenal (HPA) function in first (F1) and second (F2) generation offspring. In humans, prenatal treatment with multiple courses of sGC has been linked to behavioral disturbance in young children. We hypothesized that prenatal sGC treatment leads to altered locomotor activity and attention in young F1 offspring. Pregnant guinea pigs were treated with betamethasone (BETA; 1 mg/kg gestational days (GD) 40/41, 50/51, 60/61; n=7) or saline (Ctrl; n=8). Offspring were tested in an open field for locomotor activity on postnatal days (PND) 19 and 24, and for prepulse inhibition (PPI; PND 23) as a measure of sensory motor gating (attention). Prenatal exposure to sGC resulted in significantly increased locomotor activity in BETA male offspring in the early phases of the testing at PND19 and PND24 ($p<0.05$), and increased total activity over the 30 min test at PND24 ($p<0.05$); there was a trend towards increased total activity on PND19. There were no significant differences in locomotor activity between BETA and Ctrl female offspring. Interestingly, in both male and female BETA exposed offspring there was a significant decrease in activity over the 30 min period. In contrast, there was no significant reduction in activity in the Ctrl offspring. Prenatal sGC treatment tended to increase PPI in male offspring but decrease PPI in female offspring, though these affects failed to attain significance. Juvenile male F1 offspring exhibit increased locomotor activity in an open field and a trend towards increased prepulse inhibition (increased attention) as a result of prenatal sGC treatment. In contrast, prenatal sGC does not appear to affect activity in female offspring, though there was a trend towards reduced attention. It is evident that prenatal treatment with sGC causes significant changes in behaviors in juvenile offspring and that these effects are sex-specific. We are currently investigating the molecular mechanisms that underlie these behavioral changes as well as whether these effects extend into the next generation.
Funded by: Canadian Institutes for Health Research.

66. MECHANICAL STRETCH INDUCES THE RELEASE OF PRO-INFLAMMATORY CYTOKINES IN HUMAN MYOMETRIAL SMOOTH MUSCLE CELLS.

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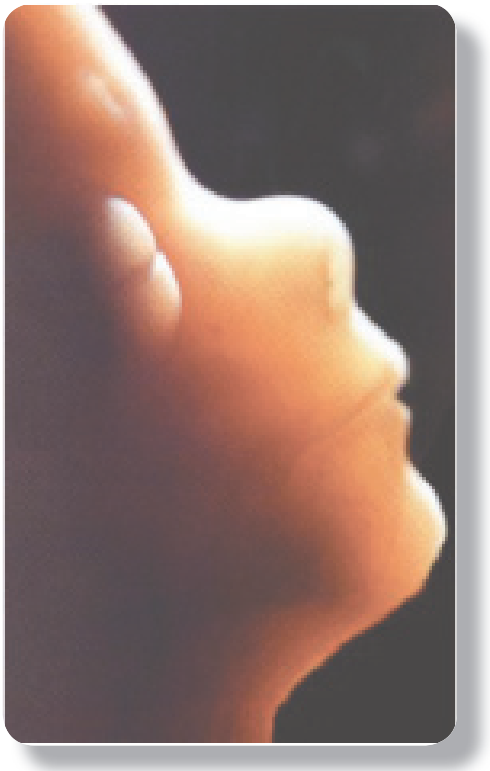
Spontaneous labour at term was found to be associated with leukocyte invasion, increased cytokine production and adhesion molecule expression. Massive infiltration of leukocytes, specifically macrophages and neutrophils, was observed in the term myometrium of pregnant women without infection, indicating their involvement in normal parturition. Our previous work demonstrating the association between uterine occupancy and the increased production of MCP-1 in term myometrium suggest that mechanical signals regulate expression of this chemokine and the initiation of labour in vivo. Concurrently, this was accompanied with an influx of macrophages in the

myometrium, which could be mediated by cytokines and adhesion molecules. We hypothesize that mechanical stretch would induce pro-inflammatory cytokine secretion by human myometrial SMCs and these cytokines would facilitate macrophage/neutrophil transendothelial migration (TEM) into the myometrium via upregulation of adhesion molecules and/or enhancement of their migratory characteristics. Methods. Human myometrial SMC line hTERT-HM were cultured on flexible-bottomed plates and subjected to static stretch for 8 and 24 hours. Culture supernatant (n=4) was collected and analyzed with Bio-Plex 27- and 21-plex human cytokine assays (Bio-Rad). With the identified cytokines, we performed TEM assay using human uterine myometrial microvascular endothelial cell line (UtMVEC-Myo) seeded onto 3-μm transwell inserts in 24-well plates to investigate whether stretch-induced cytokines promote the TEM of primary human neutrophils. Results. Preliminary Bio-Plex screen revealed a prioritized list of cytokines (VEGF, RANTES, G-CSF, IL-8, IL-10, IL-12(p70), TNF-β) whose levels were significantly elevated upon 24-hour stretch. Similar expression trend was observed for other candidates (MCP-1, GRO-α, IL-6). Preliminary TEM assay showed significant increase in neutrophil migration toward IL-8 and GRO-α stimuli in comparison to negative control. These results support our hypothesis that mechanical stretch can induce cytokine expression capable of facilitating peripheral leukocyte entry into the myometrium. Increasing number of stretch experiments and the development of transmigration assays are in progress. Funded by: Canadian Institute for Health Research - MOP- 37775

67. THE MODULATION OF ANDROGEN SIGNALING BY STEROID HORMONES AND MECHANICAL STRETCH: A NOVEL PATHWAY OF LABOR INITIATION.

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While progesterone (P4) is known to play a key role in maintaining pregnancy, androgen has also reported to inhibit myometrial contractility in pregnant women. However, little is known about the mechanisms that regulate androgen signaling during gestation. p54nrb is a co-regulator of both androgen and progesterone receptors. We hypothesized that steroid hormones and mechanical stretch of the uterus initiate labor partially by modulating androgen signaling and p54nrb expression. Objective: (1) Study androgen receptor (AR) expression of in rat myometrium during gestation and at term labor; (2) Investigate the effect of mechanical stretch and steroid hormones on myometrial AR and p54nrb expression; (3) Examine the effects of androgen signaling blockade on p54nrb expression in pregnant rat myometrium. Methods: We used rat models of (1) pregnancy and term labor, (2) unilateral pregnancy, (3) ovariectomized (OVX) non-pregnant rats primed with estrogen (E2, 10ug/kg, s.c.) and treated with/without P4 (16 mg/kg, s.c.); and (4) rats in late gestation treated with Casodex (AR inhibitor, 100 mg/kg, s.c.). Total protein and mRNA was extracted from rat myometrium and analyzed by real-time PCR and immunoblot. Results: (1) AR protein was expressed in rat myometrium in early and mid gestation and decreased significantly at term and during labor ($p<0.001$); (2) AR and p54nrb protein expression was significantly lower in gravid uterine horns versus empty horns in late gestation and during term labor (AR: $p<0.001$ and p54nrb: $p<0.05$); (3) After priming with E2, administration of P4 increased myometrial AR protein expression ($p<0.05$) and p54nrb mRNA expression ($p<0.001$); (4) Blockade of AR signaling repressed p54nrb protein expression in rat myometrium at late gestation ($p<0.01$). Conclusions: Myometrial expression of AR and p54nrb is regulated by both hormonal and mechanical signals. The decrease in androgen signaling at late gestation may represent a potential mechanism to reduce p54nrb expression and consequently initiate labor. Funded by: CIHR# 37775 and DO: PREA, MD/PhD Studentship.



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