SHERRINGTON TALKS 2019

DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

LARGE LECTURE THEATRE

SHERRINGTON BUILDING

FRIDAY 21ST JUNE

1PM

Presented by DPAG Graduate Students in their 3rd year of DPhil research study

Followed by the Departmental Happy Hour CCMN 4 –6 pm

DEPARTMENT OF PHYSIOLOGY, ANATOMY & GENETICS

UNIVERSITY OF OXFORD

SHERRINGTON TALKS 2019

Time	Speaker	Supervisor(s)	Page
1300	**Welcome address by Professor Helen Christian MD, DPAG Director of Graduate Studies**		
1305	**Nidi Tapoulal**	*A/Professor Neil Herring MD & Professor David Paterson*	3
	Neuropeptide-Y causes coronary microvascular constriction and is associated with reduced ejection fraction following ST-elevation myocardial infarction		
1315	**Rita Alonaizan**	*A/Professor Nicola Smart & A/Professor Carolyn Carr*	4
	microRNA-210-3p downregulates apoptotic cell death and mitophagy serving as a promising conditioning agent for myocardial infarction cell therapy		
1325	**Azrul Kadir**	*Dr Rhys Evans MD & Professor Kieran Clarke*	5
	Ketone body oxidation depends on anaplerosis from asparagine in isolated rat hearts lacking pyruvate precursors		
1335	**Danielle Sager**	*Professor Shankar Srinivas & Dr Andy Greenfield*	6
	Examining candidate testis determining genes		
1345	**Rosie Little**	*Dr Dominic Norris & A/Professor Mathilda Mommersteeg*	7
	Investigating the role of Nodal Vesicular Parcels and FGF signalling in left/right patterning in the mouse embryonic node		
1355	**David Dearlove**	*Professor Kieran Clarke*	8
	Exogenous ketosis: A starvation mimetic to advantageously alter human metabolism?		
1345	**Matthew Kerr**	*A/Professor Lisa Heather & Professor Damian Tyler*	9
	Energetic dysfunction in the type 2 diabetic myocardium is rescued by the deacetylase activator honokiol		
1415	**Closing Remarks & Thanks from Professor Helen Christian MD, DPAG DGS**		

Judges

Graduate Studies Committee Academics

Prize Giving following the talks

Nidi Tapoulal

Neuropeptide-Y causes coronary microvascular constriction and is associated with reduced ejection fraction following ST-elevation myocardial infarction

Supervisors: A/Professor Neil Herring MD & Professor David Paterson

Aims: The co-transmitter neuropeptide-Y (NPY) is released during high sympathetic drive, including ST-elevation myocardial infarction (STEMI), being vasoconstrictor. We hypothesized NPY levels correlate with reperfusion and recovery following primary percutaneous coronary intervention, and how NPY constricts the coronary microvasculature.

Methods & Results: Peripheral venous NPY levels were significantly ($p<0.05$) higher in patients with STEMI (n=45) compared to acute coronary syndromes/stable angina (ACS/SA, n=48) or normal coronary arteries (NC, n=16). Overall coronary sinus (CS) and peripheral venous NPY levels were positively correlated (r=0.79). STEMI patients with highest CS-NPY levels had lower coronary flow reserve, and higher index of microvascular resistance. After 48 hours they also had higher levels of myocardial edema and microvascular obstruction on cardiac MRI, and lower ejection fractions and ventricular dilatation 6 months later. NPY (100-250nM) caused significant vasoconstriction of rat coronary microvasculature, increasing vascular smooth muscle calcium waves and coronary vascular resistance in Langendorff-hearts. These effects were blocked by Y_1-receptor antagonism (BIBO3304, 1 M). NPY (250nM) also significantly increased infarct size following left coronary artery ischemia-reperfusion ($p<0.001$). Immunohistochemistry of human coronary microvasculature demonstrated presence of vascular smooth muscle Y_1-receptors.

Conclusions: High CS-NPY levels correlate with microvascular dysfunction, greater myocardial injury, and reduced ejection fraction 6 months post-STEMI. NPY constricts the coronary microcirculation via the Y_1-receptor, worsening infarct size. Y_1-receptor antagonists may be a useful PPCI adjunct therapy.

Acknowledgements also to Manish Kalla[1,2], Xi Ye[3], Lyudmyla Borysova[3], Regent Lee[2], Erica Dall'Armellina[2,4], Christopher Stanley[3], Raimondo Ascione[5], Chieh-Ju Lu[1], 'Oxford Acute Myocardial Infarction (OxAMI) Study', Adrian P. Banning[2,6], Robin P. Choudhury[2,4], Stefan Neubauer[2,6], Kim Dora[3], Rajesh K. Kharbanda[2,6], Keith M. Channon[2,6]

1Burdon Sanderson Cardiac Science Centre, Department of Physiology, Anatomy and Genetics, University of Oxford, UK.

2British Heart Foundation Centre of Research Excellence, Department of Cardiovascular Medicine, University of Oxford, UK.

3Department of Pharmacology, University of Oxford, UK.

4Oxford Acute Vascular Imaging Centre, University of Oxford UK.

5Bristol Heart Institute, Bristol Royal Infirmary, University of Bristol, UK.

6National Institute for Health Research (NIHR) Biomedical Research Centre, John Radcliffe Hospital, Oxford, UK.

Rita Alonaizan

microRNA-210-3p downregulates apoptotic cell death and mitophagy serving as a promising conditioning agent for myocardial infarction cell therapy

Supervisors: A/Professor Nicola Smart & A/Professor Carolyn Carr

Rationale and objective: Heart failure has become a leading cause of death worldwide and is most often caused by an ischemic event such as myocardial infarction (MI). Although cell therapy has provided a promising treatment for MI, the low survival of the transplanted cells in the infarcted myocardium is a limiting factor for efficacy. Therefore, the development of strategies to enhance cell survival is of extreme importance. miR-210-3p has been shown to be anti-apoptotic in multiple cell types. Here, we examine the effects of miR-210-3p on the survival and mitochondrial function of a cardiac nonmyocyte population.

Methods: Cardiac nonmyocytes were expanded from adult mouse atria as slowly adhering collagenase -trypsin cells (CTs) and characterised by immunocytochemistry. CTs were transfected with miR-210 -3p or a negative miRNA (100nM) using DharmaFECT®. CT apoptosis was assessed under serum starvation using a TUNEL assay. Gene expression was assessed by qRT-PCR, and mitochondrial copy number by mitochondrial DNA to genomic DNA ratio qPCR.

Results: CTs expressed cKit, CD44, Sca1 and Pdgfrb showing a similar expression profile to cardiosphere-derived cells (CDCs), which have been shown to improve cardiac function following transplantation in pre-clinical MI models. Upon starvation, miR-210-3p-transfected CTs demonstrated decreased apoptosis. Moreover, miR-210-3p-transfected CTs showed a downregulation in the mitophagy receptors Bnip3-like, and Pink1, enhanced mitochondrial copy number, and a downregulation in the mitochondrial fission factor Drp1.

Conclusion: Findings support an anti-apoptotic role for miR-210-3p and a novel role in regulating mitophagy presenting miR-210-3p as a promising conditioning agent for cell therapy following MI.

Acknowledgements also to J Oldbury & E smith

Azrul Kadir

Ketone body oxidation depends on anaplerosis from asparagine in isolated rat hearts lacking pyruvate precursors

Supervisors: Dr Rhys Evans MD & Professor Kieran Clarke

Aim: Myocardial ketone body oxidation in the Krebs cycle depends on anaplerosis, mainly via pyruvate from glucose and glycogen undergoing glycolysis. Inhibition of glycolysis can decrease ketone (b-hydroxybutyrate: βHB) oxidation and deplete Krebs cycle intermediates, but it is uncertain whether amino acids act as anaplerotic substrates under such circumstances. The amino acids, asparagine, valine and glutamine can be metabolised into the Krebs cycle intermediates, oxaloacetate, succinyl-CoA and a-ketoglutarate, respectively. Here, we determined whether physiological concentrations of asparagine, valine or glutamine act as anaplerotic substrates to increase βHB oxidation in isolated rat heart in the absence of pyruvate precursors.

Methods and Results: The glycogen content of isolated rat hearts was either increased or depleted by pre-perfusion with different substrates, before perfusing with buffer containing 2.5 mM glucose plus 4 mM βHB and either 0.13 mM asparagine, 0.31 mM valine or 0.65 mM glutamine. βHB oxidation was measured using [^{14}C]-βHB. In the presence of glycogen, the rate of bHB oxidation was 1.41 ± 0.25 mmoles.gww^{-1}.min^{-1}. In glycogen-depleted hearts, βHB oxidation rates were 2-fold higher on perfusion with asparagine compared to hearts perfused with 2.5 mM glucose alone (0.44 ± 0.08 vs. 0.22 ± 0.01 mmoles.gww^{-1}.min^{-1}). Neither valine (0.17 ± 0.04 mmoles.gww^{-1}.min$^{-1)}$ nor glutamine (0.22 ± 0.04 mmoles.gww^{-1}.min^{-1}) increased βHB oxidation.

Conclusion: βHB oxidation was impaired in hearts lacking adequate precursors of pyruvate-derived anaplerosis (with low glycogen and low glucose). Asparagine, an anaplerotic source of oxaloacetate, significantly increased myocardial βHB oxidation. However, anaplerosis via succinyl-CoA from valine or a-ketoglutarate from glutamine did not alter βHB oxidation.

Acknowledgements also to C Chong

Danielle Sagar

Examining candidate testis determining genes

Supervisors: Professor Shankar Srinivas & Dr Andy Greenfield

Sexual development in the mouse starts at around 10 days *post coitum* (dpc), with the formation of the fetal gonad on the surface of the mesonephros. At this stage, the gonad is a bipotential primordium. At around 11.5 dpc, the gonad acquires a fate determined by the chromosomal sex of the individual – a process known as sex determination. In XY embryos, *SRY* expression from the Y chromosome results in Sertoli cell differentiation and testis development. SRY also causes an increase in cell proliferation, which leads to an increase in size of the male gonad within 24 hours of peak SRY expression. In XX embryos, in the absence of SRY, canonical WNT signals and FOXL2 cause ovary development. The testis- and ovary-determining genetic pathways act in a mutually antagonistic fashion throughout the life of the individual to establish and maintain gonadal fate.

I am examining candidate testis-determining genes using mouse genetics approaches. Candidates are implicated by expression profile, by involvement in a biological pathway already known to be important for sex determination, or by mutations in humans with disorders of sex development (DSD). One of my candidate genes is the gene encoding the protein SEC31A, a component of the coat protein complex II (COPII). COPII mediates vesicle budding from the endoplasmic reticulum to the Golgi. It is via this pathway that many signalling molecules are transported to the cellular membrane, including WNT, a known pro-ovarian factor. Microarray data indicates that SEC31A is present in the embryonic gonadal somatic cells at the time of sex determination. The aim of this project is to discover if SEC31A has a role in testis determination and if so what part does it play in the overarching sex determination pathway. I will present phenotypic data from an analysis of a mouse *Sec31a* mutant and discuss whether it sheds light on a role for SEC31A in sex determination.

Rosie Little

Investigating the role of Nodal Vesicular Parcels and FGF signalling in left/right patterning in the mouse embryonic node

Supervisors: Dr Dominic Norris & A/Professor Mathilda Mommersteeg

Introduction

Left-right patterning is set up early in development. Cilia in the pit of the mouse left-right organiser, the node, rotate to cause leftwards fluid flow, breaking symmetry. Immotile cilia at the node edge have been proposed as mechanosensory. An alternative hypothesis suggests Nodal Vesicular Parcels (NVPs) carry signalling molecules via fluid flow (Tanaka *et al*, 2005), for detection by immotile cilia. Fgf signalling has a role in left-right patterning, (Meyers & Martin, 1998) but the precise function and mechanism is unclear.

Methods

Replicating Tanaka's protocol for live imaging of NVPs by incubation of embryos with the lipophilic dye DiI.
Investigation into expression and localisation of FGFRs in embryonic nodes and ciliated MEF cells through *in situ* hybridisation and immunofluorescence analysis.

Results & Discussion

SEM analysis of nodes show cell death and anomalous structure following incubation with DiI or mock control DMSO as per Tanaka's protocol. This casts doubt on the conclusions drawn by the use of DiI as a reagent for live imaging in this manner and the validity of NVPs as a mechanism of breaking L-R symmetry.
Specific FGFRs can be suggested to have a role in L-R patterning due to IF results showing localisation of proteins within ciliated MEFs and the mouse node itself.

David Dearlove

Exogenous ketosis: A starvation mimetic to advantageously alter human metabolism?

Supervisor: Professor Kieran Clarke

Humans have evolved to endure periods of famine. The hepatic synthesis of ketone bodies (ketogenesis) is our metabolic response to calorie deprivation. Ketogenesis helps maintain energetic homeostasis during caloric deprivation by providing a supplementary, lipid-derived fuel for brain and regulating systemic fuel selection by modulating the availability and catabolism of other substrates, including amino acids, glucose and free fatty acids. These actions significantly prolong survival during starvation and if harnessed, may offer a method to enhance human physical performance and/or provide an adjunctive therapy in the treatment of some diseases characterised by dysfunctional metabolism.

Given the availability of calorie dense food, humans in Western societies rarely enter a state of ketosis. Our laboratory has developed a ketone ester drink that elevates blood ketone levels without altering the diet. Using exercise as a metabolic stressor, my work has investigated how the unique physiological sate of exogenous ketosis affects human physiology, behaviour and metabolism and in doing so, explored ketone metabolism itself, without the confounding biochemical milieu of an endogenously induced ketosis.

Matthew Kerr

Energetic dysfunction in the type 2 diabetic myocardium is rescued by the deacetylase activator honokiol

Supervisors: A/Professor Lisa Heather & Professor Damian Tyler

Rationale/objective

Cardiovascular disease is the leading cause of mortality amongst type 2 diabetic (T2D) patients. Dysfunctional cardiac energetics, due to altered cardiac metabolism, strongly correlates with cardiovascular risk. Correcting cardiac energetics could therefore improve prognosis in T2D.

Methods/results

Energetic dysfunction was measured in perfused, contracting, T2D rodent hearts using 31P-magnetic resonance spectroscopy (MRS). T2D hearts had a 29% decrease in [ATP] and a 60% decrease in the [PCr], alongside a decrease in ATP synthesis rates measured via saturation transfer. Mitochondria isolated from T2D hearts had decreased state 3 respiration and a reduction in ATP synthesis rates, as measured by MRS on isolated mitochondria. This dysfunction was accompanied by a 51% increase in mitochondrial protein acetylation. In vitro acetylation of control mitochondria decreased respiration to the level of T2D mitochondria. In vivo administration of honokiol, a mitochondrial deacetylase activator, decreased acetylation in mitochondria from T2D rats to control levels and rescued T2D mitochondrial respiration.

Conclusion

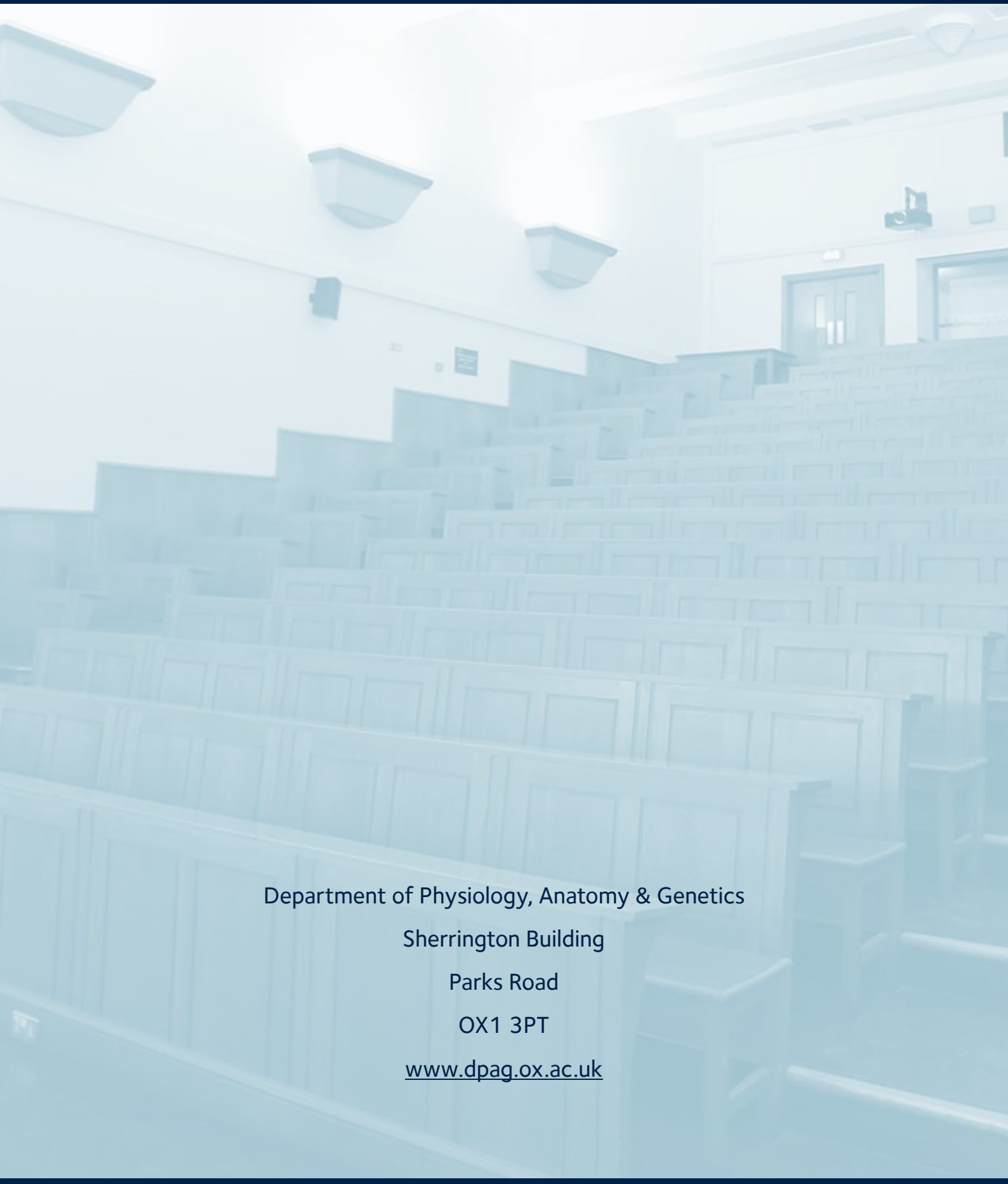
These data show that energetic dysfunction in T2D mitochondria is in part due to excessive mitochondrial acetylation, which can be pharmacologically corrected with honokiol, recovering respiratory function.

Acknowledgements also to S Stiewe, K Mehta, S Rohling and Dr J Miller

Notes

Notes

SHERRINGTON TALKS 2019



Department of Physiology, Anatomy & Genetics

Sherrington Building

Parks Road

OX1 3PT

www.dpag.ox.ac.uk