27th Northern Cardiovascular Research Group Meeting

UNIVERSITY OF LEEDS

Committee Organiser: Dr. Hannah M. Kirton

Tuesday 30th April 2019

The Great Hall and Parkinson Court

University of Leeds

Woodhouse Lane, LS2 9JT

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27th Annual Northern Cardiovascular Research Group Meeting

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Welcome to the 27th Annual Northern Cardiovascular Research Group Meeting, which is being held at the University of Leeds. We hope you find the day both beneficial and stimulating.

The Northern Cardiovascular Research Group meeting provides a stimulating arena for high calibre scientific content, informative and enjoyable discussions, collaborative opportunities and a relaxed atmosphere within a cardiovascular research community. This annual event has brought together those with an interest in the cardiovascular system since 1991 at the University of Leeds, but has moved a long way from its initial roots in the North of England, and now includes researchers from Bristol, Northern Ireland and Scotland.

The NCRG has also proudly recognised and supported students, early career researchers, and more senior researchers, to present their work to a friendly environment, providing a stimulating arena for discussion; and welcomed a New&Notable platform at the Leeds 2016 meeting.

This year we also highlight the cardiovascular research of Professor Clive Orchard (formally a University of Leeds academic member and founding organiser of this conference) with research recognition throughout the day.

Dr Hannah M. Kirton

Cardiovascular and Ion channel Postdoctorate Researcher

Sponsors

We are very grateful and honoured to receive generous support and funding from the British Heart Foundation, University of Leeds, University of Bristol and Badrilla to allow us to hold this honorary 2019 research meeting. Many thanks also to MeetInLeeds who have helped organise this meeting.

We are proud to receive support from the following:

Badrilla Mini Symposium Sponsor

We are grateful for the generous support provided by Badrilla, for today's plenary and Guest speakers.

Cairn Research New and Notable Sponsor

We are grateful for the continued and generous sponsorship from Cairn Research for the New and Notable platform sessions.

Exhibitor Sponsors

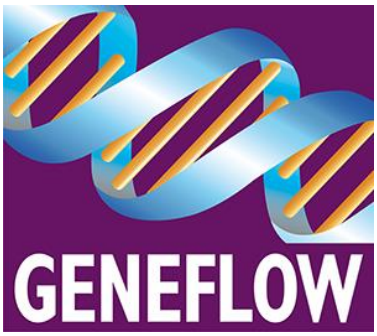
We are grateful for the generous and continued sponsorship from: ADInstruments, GeneFlow, Sarstedt, StarLab and WPI.

Gifts and Prizes

Thanks to the generous support of our local companies we are able to honour prizes and gifts at this event. Thanks to Everyman and Vue cinema, Tesco and Café Nero.

We highly encourage you to take some time to visit the exhibitors during your visit at Leeds.

The NCRG 2019 congress is kindly sponsored by



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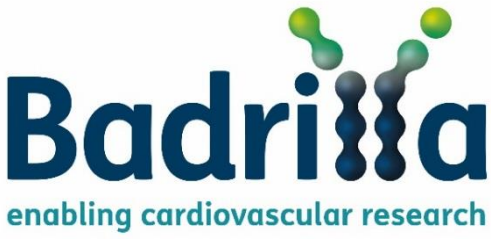


Meeting Programme 2019

Parkinson Building and The Great Hall

09.30 - 10.20 **Registration and Congress Meal deposit at The Parkinson Court front entrance**
Exhibitors/Posters
Fresh Tea/Coffee with Mini Continental Breakfast Buffet

10.20 - 10.30 **Welcome Dr. Hannah Kirton (Leeds Organising Committee)**

Session 1 The Great Hall: 10.30 – 12:00

Chairs: Dr. Matt Hardy (University of Bradford) and Ms Natasha Hadgraft (University of Salford)

10:30 – 11:00 **Cairn Research Sponsored New & Notable: Dr Izzy Jayasinghe (University of Leeds.** Presented by Martin Thomas, Author at Cairn Research

The new frontiers of optical microscopy tools for studying molecular-scale cardiac structure and function

11:00 – 11:15 **Oral Communication: Prof. Kenichi Hongo (Jikei University School of Medicine)**

Thrombin can be a novel target of the treatment of dilated cardiomyopathy

11:15 – 11:30 **Oral Communication: Dr. Parveen Sharma (University of Liverpool)**

Attenuation of doxorubicin-induced cardiotoxicity in a human in vitro cardiac model by the induction of the NRF-2 pathway

11:30 – 11:45 **Oral Communication: Dr. Olivia Robertson-Gray (University of Glasgow)**

Small molecules activating Nrf2 as a Therapeutic Approach to Prevent Cardiac Ischaemia/Reperfusion Injury

11:45 – 12:00 **Oral Communication: Dr. Riaz Akhtar (University of Liverpool)**

Unique patterns of elastin degradation in ascending aortic aneurysms in bicuspid aortic valve patients

12:00 – 13:00 **Parkinson Building: Buffet lunch with time for informal posters and exhibitors**

Session 2 The Great Hall: 13:00 – 16:00

Prof. Clive Orchard Mini Symposium

Introduction to Professor Clive Orchard
Chair: Prof. John Colyer (University of Leeds, CEO and Founder of Badrilla)

Academic Career and Research Areas

Clive Orchard BSc, PhD, DSc, is an eminent physiologist with a career-long interest in cardiac physiology. He trained at the University of London (BSc, PhD), performed post-doctoral research at University College London and the National Institutes of Health, Baltimore, and enjoyed a successful academic career at the Universities of Leeds (1986-2005) and Bristol (2005-2018). His research explored the physiology and pathophysiology of cardiac muscle contraction: calcium-signalling, acidosis, metabolic inhibition, novel therapeutics, and the role of t-tubules in excitation-contraction coupling. He created methodologies that enabled new discoveries, most notably in the area of t-tubular function.

Clive is an excellent strategist and collaborator. He is generous, collaborative, and good counsel. One of his particular strengths is the development of academic strategy for projects, groups and departments: strategies that are based fairly in the available evidence, the input from those concerned and his vision for a worthy goal. With these strengths, Clive has led the School of Biomedical Science (University of Leeds; 1998-2001), the Department of Physiology (University of Bristol; 2005-8), the Faculty of Medical and Veterinary Sciences (University of Bristol; Dean 2009-13) and the Physiological Society (President 2008-10).

Today we mark Clive's contribution to science. Beyond H-factors, money raised and papers published, Clive's contribution has been the advancement of knowledge and the development of researchers. A number of the fellows he has trained have returned to Leeds today to celebrate with Clive: some continue their interest in cardiac biology, others have branched out into new areas, but all have built upon the foundation created in Clive's lab.

Badrilla sponsors this event with delight: the company was founded on antibodies created, and first used in cardiac myocytes in collaboration with Clive in the mid-1990s. We look forward to welcoming you all to Leeds, and to celebrating together the contributions Clive Orchard has made to physiological science.

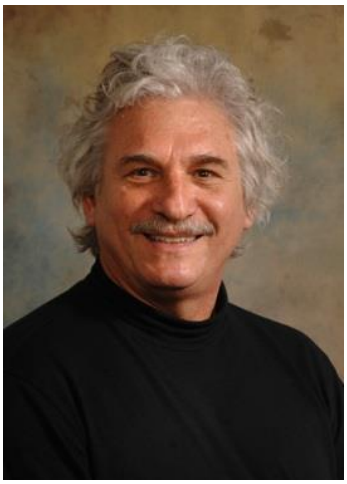
Recent Publications

- Bryant, S.M., Kong, C.H.T., Cannell, M.B., Orchard, C.H. and James, A.F. Loss of caveolin-3-dependent regulation of I_{Ca} in rat ventricular myocytes in heart failure. American Journal of Physiology 314, H521-H529. 2018.
- Bryant, S.M., Kong, C.H.T., Watson, J.J., Gadeberg, H.C., James, A.F., Cannell, M.B. and Orchard, C.H. Caveolin 3-dependent loss of t-tubular I_{Ca} during hypertrophy and heart failure in mice. Experimental Physiology 103, 652-665. 2018.
- Kong, C.H.T., Rog-Zielinska, E.A., Kohl, P., Orchard, C.H. and Cannell, M.B. Solute movement in the t-tubule system of rabbit and mouse cardiomyocytes. PNAS 115, E7073-E7080. 2018.
- Bryant, S.M., Kong, C.H., Watson, J.J., Gadeberg, H.C., Roth, D.M., Patel, H.H., Cannell, M.B., James, A.F. and Orchard, C.H. Caveolin-3 KO disrupts t-tubule structure and decreases t-tubular I_{ca} density in mouse ventricular myocytes. American Journal of Physiology 315, H1101–H1111. 2018.
- Kong, C.H., Bryant, S.M., Watson, J.J., Roth, D.M., Patel, H.H., Cannell, M.B., James, A.F. and Orchard, C.H. Cardiac-specific overexpression of caveolin-3 preserves t-tubular I_{Ca} during heart failure in mice. Experimental Physiology (ePub ahead of print). 2019

13:10 – 14:10

Badrilla Sponsored Plenary Communication

The University of Leeds and Badrilla welcome our plenary speaker Dr. Edward G. Lakatta, from the National Institutes of Health and National Institute on Aging; to the 27th Northern Cardiovascular Research Group meeting.



Dr. Edward G. Lakatta, M.D., Chief

"The Smart Heart Operates on the Edge of Criticality"

Each heart beat is initiated by spontaneous rhythmic action potentials (APs) that emanate from pacemaker cells within the sinoatrial node (SAN). Spontaneous APs in single, isolated cardiac SAN pacemaker cells are driven by a coupled-clock system of chemical and current oscillators: spatio-temporal self-organization of local Ca^{2+} releases (LCR) generates an ensemble Ca^{2+} signal during diastolic depolarization that ignites electrogenic surface membrane molecules, resulting in rhythmic oscillatory electrochemical gradient oscillations that underlie rhythmic AP cycles that determine the heart rate (HR), which varies widely across species. The coupling kinetics of the interaction of pacemaker clocks **within** single, isolated SAN pacemaker cells are self-similar **across** species from mouse, guinea-pig, rabbit to human. This pan-species self-similarity of clock-coupling kinetics in isolated SAN cells extends not only to EKG derived heartbeat intervals in vivo, but also to body mass. Thus, self-similar kinetic functions that drive rhythmic AP firing in individual pacemaker cells, to the HR in vivo, and to BM in species from mouse to human reveal novel universal scales that link microscopic subcellular mechanisms to macroscopic structural properties among diverse organisms.

Prof. Clive Orchard Mini Symposium

PART II

14:10 – 14:30 **Honorary Communication by Prof. David Eisner (University of Manchester)**

Seeing the Orchard for the trees

14:30 – 15:00 **Parkinson Building - Coffee break with informal poster session and exhibitors**

15:00 – 15:20 **Honorary Communication by Prof. Mark Boyett (University of Manchester)**

Debunking myths concerning the vagus: from 1987 to now

15:20 – 15:40 **Honorary Communication by Dr. Fabien Brette (INSERM: University of Bordeaux)**

A Calcium Odyssey

15:40 – 16:00 **Honorary Communication by Dr. Andy James (University of Bristol)**

Of mice and men: insights into the role of caveolin-3 in cardiac muscle in health and disease from genetically manipulated models.

Session 3

Parkinson Building: 16:00 – 17:00

Formal Poster Session and exhibitions

(Bespoke wine and Black Sheep beer reception)

Session 4 The Great Hall: 17:00 – 18:00

Chairs: Dr. Sandra Jones (University of Hull) and Dr. Charlotte Smith (University of Manchester)

17:00 – 17:30 **Cairn Sponsored New & Notable: Dr. Tom Claydon (SFU, Canada)**
Presented by Martin Thomas, Author at Cairn Research

Targeting hERG channel gating steps to provide cardio-protective therapies for LQTS

17:30 – 17:45 **Oral Communication: Prof. Andrew Trafford (University of Manchester)**

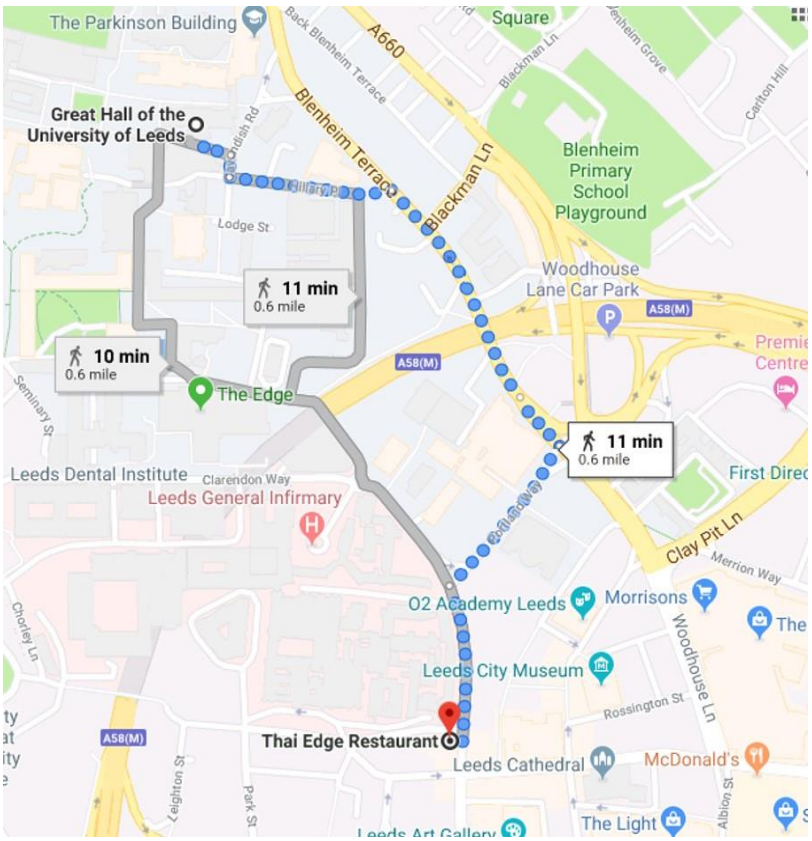
The role of protein S-nitrosylation in regulating mitochondrial function

17:45 – 18:00 **Oral Communication: Ms Florah Tshepo Moshapa (University of Bradford)**

Stabilising SOCS3 to inhibit smooth muscle cell dysfunction responsible for neointimal hyperplasia

18:00 – 18:15 **Closing remarks, prizes and announcements**

18.30 – 20:30 **Conference Three Course Buffet Meal at Thai Edge**
(with complimentary Jasmine tea or Filtered Coffee on departure)
Directions: New Portland Place, 7 Calverley Street, LS1 3DB



Abstracts (Honorary Communications)

HC1: Seeing the Orchard for the trees

Professor David Eisner, University of Manchester

Once upon a time, I arrived at University College London intending to study sodium regulation in the heart. As luck would have it, however, I found myself in the laboratory next door to where Clive Orchard was working and, together with David Allen, we studied the regulation of Ca^{2+} ions. One of our projects focused on the control of resting cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$). In particular, we investigated the effects of membrane potential, metabolism and extracellular Ca^{2+} concentration.

In this talk, I will begin with a self-indulgent review of some of my work with Clive and then fast forward 35 years to explain why I am still fascinated by the control of resting and diastolic $[Ca^{2+}]_i$. As far as I am concerned, the control of diastolic $[Ca^{2+}]_i$ and its implications for heart failure is one of the major unanswered questions in the field.

Finally, we all owe Clive an enormous debt. Today's attendees might reflect on his role in setting up the NCRG. More broadly, we are indebted to him, not only for his contributions to science but his leadership roles, both at Leeds and Bristol Universities and, nationally, as President of The Physiological Society.

HC2: Debunking myths concerning the vagus: from 1988 to now

Professor Mark Boyett, Division of Cardiovascular Sciences, University of Manchester

In 1986, Clive Orchard was recruited to the Department of Physiology at the University of Leeds. I was a young lecturer at the time, and Clive and I became close colleagues and friends. One of my best memories was in 1995 when Clive and I were awarded Personal Chairs on the same day and we celebrated with pints of beer at the Faversham pub! Clive had been working in Baltimore with Ed Lakatta and he had shown that the vagal nerve transmitter, acetylcholine (ACh), affected myofibrillar Ca^{2+} sensitivity and twitch relaxation in ferret ventricular muscle[1] and yet the dogma at the time was that the vagus only acted on supraventricular tissues in the heart. At the University of Leeds, using the jelly fish protein aequorin to measure the Ca^{2+} transient, we investigated the underlying mechanisms and showed it to be the result of the ACh-activated current and shortening of the action potential. We published the work in the *Journal of Physiology*,[2] the journal we all aspired to publish in (not *Nature*), and I was the first author, not because I did all the work, but because at this time authors were always in alphabetical order! In my career, I have continued to question the role of the vagus. We have shown that ACh affects the ventricles of various species.[e.g.3] Heart rate variability is widely used to measure cardiac vagal activity (there are >20,000 papers concerning heart rate variability), but we have shown that heart rate variability is primarily determined by heart rate and cannot be used in this way.[4] The failure of heart rate variability means that we have to reinvestigate phenomena attributed to the vagus: athletes have a low resting heart rate (~30 beats/min in elite cyclists) and this is always attributed to high vagal tone based on heart rate variability, but we reinvestigated it and found it to be dependent on a downregulation of the pacemaker channel, HCN4, in the pacemaker of the heart, the sinus node.[5,6] Based on heart rate variability, the circadian rhythm in heart rate and the low heart rate at night is attributed to high vagal tone at night, but we have shown that instead it may involve a circadian rhythm in HCN4 expression.[7]

References

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3. Yang ZK, Boyett MR, Janvier NC, McMorn SO, Shui Z and Karim F. Regional differences in the negative inotropic effect of acetylcholine within the canine ventricle. *Journal of Physiology*. 1996;492:789-806.
4. Monfredi O, Lyashkov AE, Johnsen AB, Inada S, Schneider H, Wang R, Nirmalan M, Wisloff U, Maltsev VA, Lakatta EG, Zhang H and Boyett MR. Biophysical characterisation of the under-appreciated and important relationship between heart rate variability and heart rate. *Hypertension*. 2014;64:1334-1343.
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HC3: A Calcium Odyssey

Dr. Fabien Brette, INSERM, University Hospital Bordeaux

Excitation-contraction coupling is the link between electrical activity and cell contraction. In cardiac field, the contribution from Prof. Clive Orchard is enormous. I was lucky enough to have had the opportunity to be a post-doc in his lab. This presentation highlights how working with him improved scientific career (and life). I will show some early data on the detubulation technique, which allowed me to transform from an electrophysiologist to an accomplish cardiac physiologist I will also show what happened when I became independent which lead me to a permanent position, thanks to prof. Clive Orchard. Clive is a great scientist, but also a tremendous teacher, natural leader and of course an exceptional mentor.

HC4: **Of mice and men: insights into the role of caveolin-3 in cardiac muscle in health and disease from genetically manipulated models.**

Authors: **Andrew F. James[1]**, Simon M. Bryant[1], Cherrie H.T. Kong[1], Hanne C. Gadeberg[1], Judy J. Watson[1], David M. Roth[2], Hemal H. Patel[2], Mark B. Cannell[1] & Clive H. Orchard[1].

[1]School of Physiology, Pharmacology & Neuroscience, Faculty of Life Sciences, University of Bristol, UK, [2]VA San Diego Healthcare and Department of Anesthesiology, University of California, San Diego, USA.

It is becoming increasingly apparent that cell architecture plays an important role in the regulation of contraction in the heart. Invaginations of the sarcolemma called t-tubules conduct the action potential into the cell, both ensuring co-ordinated release of Ca^{2+} from the sarcoplasmic reticulum and providing the basis for the local regulation of excitation-contraction coupling. Caveolin-3 is an ~18 kDa cholesterol-binding protein that plays an essential role in the formation of caveolae in skeletal and cardiac muscle. Caveolae are ~100 nm diameter invaginations of the sarcolemma enriched in cholesterol and sphingolipid that play important roles in cell signalling, and in membrane trafficking and composition in a wide variety of cell-types. In cardiac muscle, caveolae and caveolin-3 have been proposed to contribute to the genesis and maintenance of t-tubules and to the compartmentation of cyclic AMP signalling and regulation of L-type Ca^{2+} current at the t-tubule. Mutations in the caveolin-3 gene have been associated with familial hypertrophic cardiomyopathy and sudden cardiac death syndromes, demonstrating the importance of caveolin-3 to cardiac myocyte development and function. However, the role of caveolin-3 in the regulation of cardiac function remains unclear. This presentation summarises a series of studies that employed two genetically manipulated mouse strains, one with global knockout of caveolin-3 and the other with cardiac-specific transgenic overexpression of caveolin-3, to examine the role of caveolin-3 in the regulation of signalling at the t-tubule in health and in heart failure. The data provide evidence that loss of caveolin-3 contributes to remodelling in heart failure and ageing.

Abstracts (New & Notable)

Cairn Research Sponsored New & Notable

Dr. Izzy Jayasinghe - University of Leeds

The new frontiers of optical microscopy tools for studying molecular-scale cardiac structure and function

Izzy Jayasinghe, Miriam E. Hurley, Thomas T.M. Sheard, Kaarjel K. Narayanasamy, Alexander H. Clowsley, Michael Colman and Christian Soeller

Over the last decade, super-resolution methods known as PALM, STORM, STED and SIM have enabled better visualisation of structures and cellular signalling mechanisms in cardiomyocytes. Structures which are better-resolved include protein machineries in intracellular calcium release sites, plasmalemmal topologies (e.g. t-tubules, caveolae) and intracellular compartments. This capability is owed to an improved imaging resolution (from 250 nm to ~40 nm), albeit compromises in imaging speed (> 30 min per image), repeatability and depth imaging.

Here, we introduce a new generation of super-resolution methods which we have adapted or developed to visualise molecular-scale structures in the heart. These include DNA-PAINT which has allowed the repeated visualisation and 'counting' of single proteins at an unprecedented resolution of 10 nm. We developed "Enhanced Expansion Microscopy" (EExM) which allowed *in-situ* identification of the three-dimensional position and phosphorylation state of individual ryanodine receptors (RyRs) within cardiomyocytes. A new imaging method developed by us, called 'sandSTORM', uses a new generation of label probes to both accelerate (by a factor of ~ 5) and sustain long-term imaging (>10 hours continuously) of proteins such as RyRs.

We have used these image data to characterise the nanometre-scale reorganisation and spatially-heterogeneous phosphorylation of RyRs in ventricular myocytes in a model of right ventricular failure. By incorporating the experimentally-mapped positions and chemical signatures of RyRs into computational models, we are now able to predict (i) the likely mechanisms of RyR cluster self-assembly *in situ* and (ii) the nanometre-scale evolution of calcium signalling patterns resulting from the structural and chemical remodelling.

Cairn Research Sponsored New & Notable

Dr. Tom Claydon – Simon Fraser University, Canada

Targeting hERG channel gating steps to provide cardio-protective therapies for LQTS

Patrick Shi, Samrat Thouta, Christina Hull, May Cheng, Jacob Kemp, Kyle Simpson, Ravichandra V, Shoaib Faizi, Zhaokai Pang, Raj Johal, **Tom Claydon**

Cardiac potassium channels contribute to repolarization of the cardiomyocyte action potential. Their functional diversity produces heterogeneous repolarization in different regions of the heart, which contributes to the orchestration of rhythmic excitation of the myocardium. This diversity arises from variations in biophysical gating mechanisms, which produces markedly different channel behaviours. Cardiac Kv11.1 potassium channels are of particular interest, because they have unique and unusual gating, their dysfunction predisposes sudden cardiac death, and their high affinity for a diverse range of pharmacological compounds presents a significant challenge to pharmaceutical drug development. We have used conventional and fluorescence-based electrophysiological approaches to study potassium channel gating mechanisms. Measuring ion current flow through the channel pore and dynamics of the voltage sensing domain in Kv11.1 channels, we have characterized important gating mechanisms and explored their molecular determinants. Using optical mapping of *ex vivo* whole zebrafish hearts, we highlight the role of these biophysical events in the protection against cardiac arrhythmias and suggest potential novel therapeutic strategies for ameliorating the effects of inherited channel dysfunction.

Abstracts (Oral Communications)

OC1: **Thrombin can be a novel target of the treatment of dilated cardiomyopathy**

Authors: Kenichi Hongo, Makoto Kawai, Kimiaki Komukai

The Jikei University School of Medicine

Introduction: Hypercoagulability state has been observed in patients with dilated cardiomyopathy (DCM) compared to healthy subjects. In addition to being found in blood, thrombin is also expressed in the heart. Therefore, thrombin in the heart tissue may contribute to the pathophysiology of DCM.
Purpose: This study aimed to investigate whether tissue thrombin expression is associated with the pathogenesis of DCM.
Methods: We evaluated the expression of thrombin by immunohistochemical analysis in the left ventricle of 5 patients with DCM undergoing Batista operation and that of 4 patients without heart disease serving as control. The immunohistochemical staining was scored subjectively on a semi-quantitative scale of 0–4. We investigated the effects of the direct thrombin inhibitor, dabigatran, in the development of DCM in a mouse model carrying a deletion mutant of cardiac troponin T (DCM mouse) which causes human DCM, by using echocardiography and Kaplan-Meier method. We also estimated the apoptotic index using the terminal dUTP nick-end labeling (TUNEL) assay using the heart tissue from DCM mouse.
Results: Immunohistochemical analysis showed strong thrombin expression in DCM patients compared to patients without heart disease. Dabigatran significantly improved impaired cardiac function of DCM mouse by echocardiographic examination. Dabigatran also rescued poor outcome of DCM mouse. The apoptotic index in DCM mouse heart was significantly reduced by dabigatran treatment.
Conclusion: Upregulation of tissue thrombin might be involved in the pathogenesis of DCM. The direct thrombin inhibitor, dabigatran, possibly be a treatment option against DCM.

OC2: **Attenuation of doxorubicin-induced cardiotoxicity in a human in vitro cardiac model by the induction of the NRF-2 pathway**

Authors: Lauren Tomlinson, Zhen Qi Lu, Robert A Bentley, Helen E. Colley, Craig Murdoch, Steven D. Webb, Michael J. Cross, Ian M. Copple, **Parveen Sharma**

University of Liverpool

Dose-dependent cardiotoxicity is the leading adverse reaction seen in cancer patients treated with doxorubicin. Currently, dexrazoxane is the only approved drug that can partially protect against this toxicity in patients, however, its administration is restricted to those patients receiving a high cumulative dose of anthracyclines. Investigations into the mechanisms of cardiotoxicity and efforts to improve cardioprotective strategies have been hindered by the limited availability of a phenotypically relevant in vitro adult human cardiac model system. In this study, we adapted a readily reproducible, functional 3D human multi-cell type cardiac system to emulate patient responses seen with doxorubicin and dexrazoxane. We show that administration of two NRF2 gene inducers namely the semi-synthetic triterpenoid Bardoxolone methyl (CDDO-me), and the isothiocyanate sulfurophane, result in cardioprotection against doxorubicin toxicity comparable to dexrazoxane as evidenced by an increase in cell viability and a decrease in the production of reactive oxygen species. We further show a synergistic attenuation of cardiotoxicity when the NRF2 inducers and dexrazoxane are used in tandem. Taken together, our data indicate that the 3D spheroid is a suitable model to investigate drug induced cardiotoxicity and we reveal an essential role of the NRF2 pathway in cardioprotection providing a novel pharmacological mechanism and intervention route towards the alleviation of doxorubicin-induced toxicity.

OC3: **Small molecules activating Nrf2 as a Therapeutic Approach to Prevent Cardiac Ischaemia/Reperfusion Injury**

Authors: Olivia Robertson-Gray, Alexandra Riddell, Albena Dinkova-Kostova, William Fuller.

University of Glasgow

Myocardial ischaemia/reperfusion (I/R) injury occurs via several mechanisms including the production of reactive oxygen species (ROS) upon reperfusion. The transcription factor, Nrf2, regulates cytoprotective processes associated with detoxification of ROS. Under homeostatic conditions, Nrf2 is targeted for ubiquitination and proteosomal degradation by Keap1, keeping cytoplasmic levels low. In response to electrophiles which chemically modify Keap1, Nrf2 ubiquitination is inhibited, causing it to accumulate and increase transcription of its protective target genes. RTA dh404 is an established activator of Nrf2 target genes and may represent a clinically useful strategy to reduce I/R injury. Methods: Mice were dosed with dh404 (10-50mg/kg; oral gavage) once daily for 3 days with experiments taking place 24 hours after the final dose. RNA was extracted from pulverised hearts, reverse transcribed, and cytoprotective target gene / housekeeping gene abundance assessed by qPCR. In separate experiments, hearts were perfused in Langendorff mode, subjected to a 30-minute ischaemia/40-minute reperfusion protocol where indices of cardiac function were measured. Hearts were then frozen, sectioned and stained with TTC to delineate infarcted tissue, the area of which was measured via computerised planimetry. Results: dh404 (30-50mg/kg) increased the expression of cytoprotective target genes within the heart (P<0.05). Isolated heart experiments revealed that dh404 (30mg/kg) preserved inotropy and lusitropy (P<0.05) and increased left ventricular developed pressure (LVDP) during reperfusion (P>0.05). Infarct size was also significantly reduced (P<0.01). Conclusion: oral dh404 increases the expression of cardiac cytoprotective genes, ameliorates cardiac dysfunction during the reperfusion period and reduces myocardial I/R injury.

OC4: **Unique patterns of elastin degradation in ascending aortic aneurysms in bicuspid aortic valve patients**

Authors: Ya Hua Chim[1], Hannah Davies[2], Mark Field[3], Jill Madine[2], **Riaz Akhtar[1]**

[1] Department of Mechanical, Materials and Aerospace Engineering, University of Liverpool, Liverpool, L69 3GH, [2] Institute of Integrative Biology, University of Liverpool, Liverpool, UK [3] Liverpool Heart and Chest Hospital, Liverpool, UK

Bicuspid aortic valve patients (BAV) are associated with increased risk of ascending aortic aneurysms. However, is unclear whether matrix degradation varies in different ascending aneurysm aetiologies. Here, we measured the micromechanical and biochemical properties and characterised elastin microstructure within the aortic tissue of two specific aneurysmal groups; BAV with associated aneurysm (BAV-A) and idiopathic degenerative aneurysm (DA). Aneurysmal tissues are compared against control tissue.

Methods: Aortic tissue was obtained from patients undergoing aneurysmal repair surgery (BAV-A; n=15 and DA; n=15). Control tissue was punch biopsies obtained during coronary artery by-pass graft (CABG; n=9). The elastic modulus (E) was measured with nanoindentation for the medial layer. Glycosaminoglycan (GAG), collagen and elastin levels were measured using biochemical assays. Verhoeff Van Gieson-stained sections were imaged for elastin microstructural quantification.

Results: BAV-A had over 20% higher E relative to control and DA. No significance between DA and control due to tissue heterogeneity. Collagen level of BAV-A (36.9±7.4μg/mg) and DA (49.9±10.9μg/mg) was higher compared to the control (30.2±13.1μg/mg). GAG and elastin levels were not significant between the groups. Elastin segments were uniform in controls. Aneurysmal tissues had loss of segments close to the intima and adventitia layers. Although BAV-A and DA had more elastin segments compacted in the media, elastin segments were highly fragmented in DA.

Conclusions: BAV-A has increased stiffness within the aortic wall relative to DA and control tissue. Although elastin levels were equal for all groups, spatial distribution of elastin provided us with a unique profile of matrix degradation for BAV-A.

OC5: **The role of protein S-nitrosylation in regulating mitochondrial function**

Authors: E. J Radliffe[1], J Sun[2], E Murphy[2], M. Murphy[3], G Galli[1], **A Trafford[1*]**

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Introduction: Heart failure is associated with a loss of cardiac contractility and an increase in nitric oxide production. The electron transport chain is the primary source of ATP for cardiac contraction and has previously been shown to be inhibited by S-nitrosylation following ischemia. However, relatively little is understood about the role of this modification in chronic heart failure.

Methods: This study used an ovine model of heart failure. S-nitrosylation was enriched for using resin assisted capture. Mitochondria were isolated for oxygraph experiments.

Results: Heart failure resulted in loss of left ventricular contractility and an increase in the number of S-nitrosylated proteins. Several electron transport chain subunits had increased levels of S-nitrosylation without corresponding changes in abundance. The addition of a mitochondrially targeted nitic oxide donor (mitoSNO) to control mitochondria inhibited pyruvate, malate and glutamate driven state 3 respiration. MitoSNO inhibition was reversible on the application of 1mM DTT but not by oxyhaemoglobin suggesting that this was an effect of S-nitrosylation as opposed to free nitric oxide.

Conclusions: This study demonstrates that heart failure is associated with an increase in myocardial and mitochondrial S-nitrosylation. S-nitrosylation of the electron transport chain inhibits respiration and thereby ATP production and thus contributes to the progressive loss of function observed in heart failure.

OC6: **Stabilising SOCS3 to inhibit smooth muscle cell dysfunction responsible for neointimal hyperplasia**

Authors: Florah T. Moshapa[1], Jamie J.J.L. Williams[2], Jacobo Ellies[1], Kirsten Riches-Suman[3], Timothy M. Palmer[4].

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Suppressor of cytokine signalling 3 (SOCS3) limits JAK/STAT pathways involved in vascular inflammation and remodelling responsible for vein graft failure. However, SOCS3 is limited by its short biological half-life. We hypothesise that a stabilised “Lys-less” SOCS3 may have greater therapeutic potential than wild type (WT) in limiting JAK/STAT-mediated processes responsible for neointimal hyperplasia in type 2 diabetes mellitus (T2DM).

Smooth muscle cells (SMCs) and endothelial cells (ECs) isolated from human saphenous vein (HSV) were transduced with recombinant lentiviruses, MOI=3.6 (WT), 22.2 (Lys-less SOCS3) and 5.6 (GFP) tu/cell. Successful transduction was confirmed by immunofluorescence and immunoblotting. Ubiquitylation was tested by immunoprecipitation and immunoblotting. SOCS3 stability was determined by emetine chase. HSV-SMC proliferation (cell counting and CyQuant assay) and migration (Boyden chamber) were also assessed. Finally, SOCS3 effects on signalling were assessed by measuring phosphorylation of STAT3 (Tyr705) and ERK1/2 (Thr202/Tyr204) by immunoblotting.

Lentivirus transduction of WT and Lys-less SOCS3 in HSV-SMCs and ECs was highly efficient after 48h (n=4) and sustained for at least 2 weeks. Lys-less SOCS3 was resistant to ubiquitylation contrary to WT transduced HSV-ECs (n=3). Lys-less SOCS3 was also more stable (t1/2=4h) than WT (t1/2<4h) (n=6). Concomitant with a reduction in sIL-6Rα/IL-6-induced proliferation in transduced HSV-SMCs, WT and Lys-less SOCS3 specifically inhibited sIL-6Rα/IL-6-mediated STAT3 activation (n=5). WT also blocked PDGF-BB-induced STAT3 activation (n=3).

In summary, Lys-less SOCS3 can be successfully transduced into HSV-SMCs and ECs for at least 2 weeks. Lys-less SOCS3 is stable and ameliorates the adverse effects of inflammatory mediators on cell function and signalling.

Abstracts (Posters)

Arranged in order of presenting author's surname

P1: Identification of key transcription factors in the adult human sinus node

Authors: Abimbola J Akerele, Maria Petkova, Joseph Yanni, Andrew J. Atkinson, Peter Molenaar, Filip Perde, Delvac Oceandy, Alicia D'Souza, Halina Dobrzynski
University of Manchester

The sinus node (SN) is the heart's pacemaker. Its myocytes are small, embedded in connective tissue. The SN expresses ion channels (e.g., HCN4, $Ca_v3.1$) important for its function. Developmental transcription factors (TFs) such as Tbx3, Tbx5, Tbx18, Shox2, Nkx2-5 and Isl1 regulate these molecules during embryogenesis. It is known that developmental TFs are re-employed in diseased adult heart (e.g., Nkx2-5). Therefore, our aim was to identify TFs in the adult human SN.
Three SN/right atrium (RA) specimens were obtained from healthy human hearts with appropriate ethical approval. Histology and immunofluorescence identified the SN by abundance of connective tissue and HCN4 expression. RNA was isolated from the SN and its surrounding RA. Next generation sequencing was performed and Ingenuity Pathway Analysis was used to identify TFs in our datasets. Mean data (SN, n=3 and RA, n=3) revealed 68 significantly more (e.g., Isl1, Shox2, Tbx3 and Tbx18; log2 fold change>1, $p<0.05$) and 60 significantly less (e.g., Tbx5 and Nkx2-5; log2fold change<1, $p<0.05$) in the SN vs. RA. Novel TFs were also identified in the SN (e.g., HOXA5, HOXB2, HOXB3, HOXB4, HOXD8, HOXC4, DLX2, PHOX2B, VENTX).
We show that developmental TFs are retained in the adult human SN. We also show novel TFs in this tissue. Next, we aim to determine function of novel TFs and if they are remodelled in diseased SN in aid to develop better therapies to treat sick sinus.

P2: Investigation of blood brain barrier damage in a murine model of Stroke

Authors: Meaad Almusined, Nadira Y Yuldasheva, Sikha Saha
University of Leeds

The blood brain barrier (BBB) is a specialized structure separating the brain from the peripheral blood circulation and plays an important role in maintaining the microenvironment for normal brain function and signalling. BBB disruption remains an important complication of ischaemic stroke and a major determinant of stroke outcome in patients. The BBB is formed by brain microvascular endothelial cells interconnected by tight junctions, which selectively exclude most blood-borne substances from entering the brain. Pericytes and astrocytes are critical for maintaining normal BBB physiology and function. However less is known about cerebral ischemia and reperfusion induced BBB damage. The aim of this study is to examine the changes in BBB cells and tight junction proteins in in a mouse model cerebral ischaemia and reperfusion to further understand the mechanisms of stroke and to develop new therapeutic approaches targeting the BBB.

P3: Calcium signalling in the endothelium and control over vascular tone in health and obesity.

Authors: Mariam Alakrawi, Adam Greenstien
University of Manchester

The endothelium of the small resistance artery induces vasodilation. Mechanisms underlying endothelial- dependent relaxation are dependent on brief localized changes in intracellular [Ca^{2+}]. The predominant Ca^{2+}events underlying these vasodilatory responses are: Ca^{2+} pulsars, which are IP3-mediated, and Ca^{2+} sparklets that reflect Ca^{2+} entry through TRPV4 channels. Generation of both Ca^{2+} pulsars and Ca^{2+}sparklets initiate Ca^{2+} induced activation of intermediate (IK) and small conductance (SK) channels in the endothelium, causing hyperpolarization of the whole artery and subsequent vascular relaxation. As such, the process of Ca^{2+} events coupling to Ca^{2+} - sensitive K^+ channels is known as 'Endothelium Dependent Hyperpolarization' (EDH). In human small arteries, obesity is associated with severe dysfunction of the endothelium, manifest as a failure in vasodilation to stimuli such as muscarinic agonists. The mechanisms underlying this dysfunction are unclear, particularly in terms of any changes to regulation of the aforementioned Ca2+ events and the vasodilatory ion channels. The objective of this study is to quantify the changes to endothelial Ca2+ signaling in human obesity and relate this to the vasodilatory capacity of the endothelium. Methods and Results: human resistance omental arteries from pregnant women at cesarean delivery were used. Wire myography assessed wire-induced stretch and agonist effects. Endothelial Ca2+ pulsars and Ca2+sparklets were studied using high speed spinning disc confocal microscopy. This study demonstrated that TRPV4 and IK channels dose dependably relax U46619 preconstricted omental artery segments, and that Ca2+ pulsars are present in human endothelium. Conclusion: The TRPV4- IK signaling pathway causes human omental artery relaxation through EDH.

P4: Variation in cardiac long non-coding RNAs in congenital heart disease patients

Authors: Stephanie Baross, Simon Williams, Kathryn Hentges, Andrew Sharrocks, Bernard Keavney
University of Manchester

Congenital heart disease (CHD) is the most common birth defect affecting 1% of live births. CHD shows a high degree of heritability with many of the known causative genes having roles in gene regulation. However, the genetic causes of CHD are still poorly understood overall. One possible explanation for the "missing heritability" of CHD is long non-coding RNAs (lncRNAs), which often act as regulators of gene expression and are excluded from exome-focussed studies. Previous studies have identified multiple lncRNAs with roles in regulation of heart development but these lncRNAs have not been studied in CHD patients to determine if they have a causal role. We have used whole genome sequencing data from 660 CHD patients in the 100,000 Genomes Project to identify variants in six cardiac lncRNAs. Variants in all six tested lncRNAs were significantly enriched ($p<0.0001$) in CHD patients compared to the control group, with between 8 and 10 times more variants seen than expected. Common variants were removed by filtering against gnomAD (genome aggregation database) and 52,513 control genomes from individuals in the 100,000 Genomes database who do not have CHD or related disorders to generate a list of variants unique to CHD patients. The identified CHD-unique variants will be cross-referenced with eQTL data and predicted secondary structures to prioritise variants which are likely to affect lncRNA function. Prioritised variants will be modelled in suitable *in vitro* models to measure the effects of the variants on lncRNA function and heart development pathways.

P5: Make your western blot P.A.S.S.: AbQuant calibration generates more robust quantitative data and improves productivity

Authors: Richard J. Bennett[1,3], Moninder S. Bhogal[1], Ruth Norman[2], Sarah Callaghan[2], Victoria Harman[3], Al Benson[2], Rob Beynon[3], John Colyer[1,2]
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Western blotting is a ubiquitous technique in biological research, often used for protein quantification. However, the assay is often performed without full validation, potentially compromising the reliability of the data. To address this this we have developed the P.A.S.S. framework for assay validation; requiring consideration of assay **P**recision, **A**ccuracy, **S**pecificity & **S**ensitivity. Here we evaluate the precision of western blotting, whether precision can be improved by the incorporation of appropriate protein calibration standards and the impact improved precision has on sample size required for a sufficiently powered experiment.

We have developed a multiplexed calibration technology (AbQuant) for immunoblot application. We deployed AbQuants into either cardiac myocyte lysate or rat heart tissue homogenate and loaded panels of technical replicates three times across a single gel.

The AbQuant calibration curve was used to convert raw analyte signal into an absolute unit. Variance calculated using raw signal from technical replicates exhibited poor precision (average CV, coefficient of variation, 28.7%, n=72), whereas after calibration variance decreased (average CV 17.2%). We modelled how this reduction in variance would affect the required sample size at 80% power, 50% effect size and 5% significance threshold. On average, across eight experiments, the required sample size was reduced by 50% when calibration was used.

We have demonstrated that western blots are inherently variable, making quantitative conclusions difficult. The addition of an AbQuant calibration standard improves precision significantly and decreases the sample size required for a sufficiently powered study. This addresses both reliability of research observations and the productivity of research programmes.

P6: **Investigating the role of KMT2C in tetralogy of Fallot**

Authors: Shona Borland, Gennadiy Tenin, Simon Williams, Richard Monaghan, Matthew Baxter, David Ray, Sabu Abraham, Bernard Keavney
University of Manchester

Congenital heart disease describes a group of defects resulting from aberrant heart development. The causes of congenital heart disease are poorly understood. Histone modifying genes have previously been implicated in congenital heart disease. *KMT2C* is a histone modifying gene which contains a catalytic SET domain which methylates histone 3 lysine 4 regulating gene expression. We have previously identified 18 mutations in *KMT2C* in a whole-exome sequencing study of 829 non-syndromic tetralogy of Fallot patients. These mutations are rare (absent in GnomAD) and likely deleterious (CADD score>20). We have now investigated the role of this gene in heart development further. Firstly we have studied *KMT2C* expression using RT-PCR and *in situ* hybridisation. *Kmt2c* is expressed throughout the mouse embryonic heart from embryonic day 11.5 to 14.5 which is the key developmental time period for heart development with respect to the defects observed in tetralogy of Fallot. In human embryonic hearts expression of *KMT2C* was also found at equivalent stages (between Carnegie stages 13 and 20). Using a mouse model where the SET domain of KMT2C has been deleted, we have investigated the role of KMT2C in heart development. In mouse embryos homozygous for the deletion, all embryos appear to have abnormal heart development. Ventricular septal defects with or without an over-riding aorta are the most common defect indicating similarities to tetralogy of Fallot. This work demonstrates that *KMT2C* is a good candidate gene for tetralogy of Fallot, both due to its cardiac expression and the defects identified in mouse studies.

P7: **Disordered yet functional atrial t-tubules on recovery from heart failure.**

Authors: Jessica L. Caldwell, Jessica D. Clarke, Christian Pinali, David A. Eisner, Andrew W. Trafford & Katharine M. Dibb.

University of Manchester

Transverse (T)-tubules are vital for the synchronous rise of systolic calcium. Heart failure (HF) is commonly associated with t-tubule loss leading to dyssynchronous calcium release. Recovery from HF is associated with t-tubule restoration and normalisation of systolic calcium. The mechanisms that control t-tubules remain unknown, thus we aim to determine; i) if atrial t-tubule loss in HF can be recovered, ii) the effect of t-tubule restoration on systolic calcium, iii) proteins involved in t-tubule recovery.

HF was induced in sheep by rapid ventricular pacing. Rapid pacing lead to loss of virtually all atrial t-tubules and the initial rise of calcium was restricted to the surface sarcolemma. Cessation of pacing resulted in recovery of cardiac function and recovery of atrial tubule density to control values. Furthermore, when loaded with Fluo-8AM, calcium was firstly released along the tubules in the recovered myocytes, followed by propagation to the rest of the cell. Expression of BIN1, Tcap and MTM1 correlated with t-tubule density. Vectors encoding BIN1, Tcap and MTM1 were transiently expressed in neonatal rat ventricular myocytes. After 48hrs, overexpression of BIN1 led to the development of tubules, the structure of which was altered by coexpression with MTM1 and Tcap.

In conclusion, atrial t-tubules are lost in a sheep model of HF; this is associated with dyssynchronous calcium release. T-tubule associated proteins BIN1, MTM1 and Tcap also decrease during heart failure. Following recovery from HF t-tubule density, alongside calcium transient amplitude, is restored which could be due to up regulation of these proteins.

P8: **Nanomechanical and nanostructural properties of collagen in ascending aortic aneurysm**

Authors: Chim, Y.H.[1], **Davies, H.[2], Field, M.[3], Madine, J.[2], Akhtar, R.[1]**

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Introduction: Degradation of collagen is an important pathway related to aortic aneurysms. However, it is unclear whether collagen properties differ in different aneurysm aetiologies. Here, we measured the nanomechanical properties and characterised collagen fibres in the aortic tissue of two specific groups; bicuspid aortic valve with associated aneurysm (BAV-A) and idiopathic degenerative aneurysm (DA). **Methods:** Aortic tissue was retrieved from 14 age-matched patients undergoing either BAV-A or DA aneurysmal repair. Atomic force microscopy (AFM) was used to characterise the collagen fibres within the medial layer; the elastic modulus (*E*) and deformation was obtained. Captured AFM images (n=4 per patient) were used to quantify the collagen fibres; the fibre diameter and collagen d-periodicity was measured. **Results:** *E* of BAV-A was found to be significantly higher than DA (p=0.007). Expectedly, deformation was significantly lower in BAV-A relative to DA (p=0.01). DA was found to have larger collagen fibre diameter and d-periodicity compared to BAV-A. When the collagen properties were compared with *E*, a positive correction was found in both aneurysmal tissues. However, when collagen diameter was correlated with deformation, opposite trends were observed for BAV-A and DA; negative and positive respectively. **Discussion:** BAV-A tissue was significantly different to DA tissue, having shorter fibre properties and higher tissue stiffness. Interestingly, depending on the type of aneurysm the collagen fibre diameter correlates differently with its nanomechanical properties. These initial observations provide new insight to ascending aortic aneurysms.

P9: **Investigating the role of Pak2 in the heart following myocardial infarction**

Authors: Lucy Collins, Pablo Binder, Wei Liu, Xin Wang

University of Manchester

Myocardial infarction (MI) and hypertension related disease, such as cardiac hypertrophy, are two principal causes of heart failure (HF), for which the prognosis has not improved in 20 years. Evidently, therapies to prevent and treat HF are required. Pak2 has been shown to localise near to the ER membrane and promote a cardioprotective ER stress response during cardiac hypertrophy, resulting in reduced cardiomyocyte death and improved cardiac function. This project aims to assess whether Pak2 has a similar role in the heart following MI. Pak2 cardiac knockout mice ($Pak2^{cko}$) are used to investigate Pak2's role *in vivo*. Due to the high mortality of $Pak2^{cko}$ and their $Pak2^{f/f}$ littermates following MI, a less severe MI model, which involves ligating the lateral anterior descending artery closer to the apex of the heart, has been verified and will be used as the MI model for this project. Preliminary data has shown that Pak2 becomes activated in the acute MI response *in vivo*, as well as following hydrogen peroxide-induced oxidative stress *in vitro*. To assess Pak2's mechanism of action, an adenovirus overexpressing constitutively active Pak2 has been generated and tested on adult rat cardiomyocytes. This will be used alongside an adenovirus expressing short-hairpin Pak2, leading to Pak2 knockdown, to investigate Pak2's mechanism *in vitro*.

P10: **Arrhythmia from dyad to whole-heart: bi-directional coupling between re-entry and spontaneous calcium release**

Authors: Michael A. Colman
University of Leeds

The mechanisms underlying the initiation and perpetuation of cardiac arrhythmias are inherently multi-scale: whereas arrhythmias are intrinsically tissue-level phenomena, they have a significant dependence cellular electrophysiological factors. Spontaneous sub-cellular calcium release events (SCRE), such as calcium waves, are exemplars of the multi-scale nature of cardiac arrhythmias: stochastic dynamics at the nanometre-scale can influence tissue excitation patterns at the centimetre scale, as triggered action potentials elicit focal excitations. This has been long proposed as a mechanism underlying the initiation of rapid arrhythmias such as tachycardia and fibrillation, yet systematic analysis of these multi-scale interactions is lacking. Moreover, potential bi-directional coupling has been seldom explored even in concept.

A major challenge of dissecting the role and importance of SCRE in cardiac arrhythmias is that of experimentally simultaneously exploring sub-cellular and tissue function. Computational modelling provides a potential approach to perform such analysis, but requires new techniques to be employed to practically simulate sub-cellular stochastic events in tissue-scale models comprising thousands or millions of coupled cells.

This presentation will outline the novel techniques developed to achieve this aim, and explore preliminary studies investigating the mechanisms and importance of SCRE in tissue-scale arrhythmia: How do independent, small-scale sub-cellular events overcome electrotonic load and manifest as a focal excitation? How can SCRE focal (and non-focal) dynamics lead to re-entrant excitation? How does long-term re-entrant excitation interact with SCRE to perpetuate and degenerate arrhythmia?

P11: **Palmitoylation and the regulation of the "funny" current HCN411**

Authors: Samitha Congreve, Dr Fiona Plain, Dr Will Fuller, Professor Jules Hancox
University of Glasgow

S-palmitoylation regulates key cardiac Na+ and Ca2+ handling proteins, influencing their membrane microdomain localisation and function. The cardiac conduction system involving the sinoatrial node (SAN) generates coordinated rhythmic action potentials propagating to the rest of the heart, and is essential for the generation of the heartbeat. Various ion channels contribute to the spontaneous activity of the SAN, including the hyperpolarisation-activated cyclic nucleotide-gated channel HCN4. The HCN4 is responsible for the "funny" pacemaker current (If), a key component of the "membrane clock". HCN4 channels localise to lipid rafts and disorganisation of rafts results in redistribution of the channels, altering its kinetic properties. S-palmitoylation as the only dynamic reversible lipid modification, acts as a mechanism of targeting transmembrane proteins and ion channels into lipid rafts. This project will adopt an in vitro approach to determine the biochemical and biophysical characterisation of HCN4 palmitoylation and its functional consequences. We have successfully mapped the primary palmitoylation sites on HCN4, established the impact of thioesterase inhibitors and expressing caveolar coat proteins on HCN4 palmitoylation. Furthermore, we will identify DHHC-PAT enzyme isoforms that mediate HCN4 palmitoylation, quantify effects of palmitoylation on HCN4 function and explore whether SAN HCN4 palmitoylation is altered in a heart failure model. The results will provide extensive new information on hitherto unstudied post-translational regulation of a key cardiac pacemaker ion channel.

P12: **Diet induced obesity leads to impaired mitochondrial dynamics in the heart**

Authors: Hussam Daghistani, Sophie Saxton, Sukhpal Prehar, Min Zi, Elizabeth Cartwright and Ashraf Kitmitto
University of Manchester

Background: The development of cardiac mitochondrial dysfunction occurs in the early stages of type 2 diabetes, T2DM. However, there is a gap in knowledge surrounding the mechanisms underpinning changes to mitochondrial function, particularly dynamics, as a result of obesity and/or T2DM. This project aims to i) characterise cardiac function and investigate mitochondrial fission/fusion mechanisms in a diet-induced murine model of obesity and ii) determine whether introducing an exercise regimen can improve cardiac and mitochondrial function. **Methods:** Eight weeks old C57BL/6J male mice were fed with either 60% HFD or chow (control) for 12 weeks. A second HFD group was established incorporating exercise training starting at week 12 for 5 weeks (whilst still maintained on a HFD) to determine the impact upon cardiac and mitochondrial function. **Results:** HF feeding led to weight gain, hyperglycaemia and insulin resistance with indications of early LV dysfunction. Molecular analyses showed increases to the expression of the inner mitochondrial fusion protein Opa1 and fission related proteins Drp1 and Fis1 ($p \leq 0.05$). While exercising training led to weight loss the mice remained insulin resistant with alterations to the fission-fusion protein axis. **Conclusion:** Our results showed an imbalance to mitochondrial dynamics occurs in the murine model of diet-induced obesity. Opa1 may be cardioprotective to stabilise cristae structure since there was no change to the proteins regulating fusion of the outer mitochondrial membrane. The increase in Drp1 and Fis1 would indicate mitochondrial fragmentation. While exercise led to a reduction in heart rate and improvement to cardiac function, at the cellular level mitochondrial dynamics remained perturbed.

P13: **Idiopathic degenerative thoracic aneurysms are associated with increased aortic medial amyloid**

Authors: [a]**Hannah A. Davies**, [b]Eva Caamaño-Gutiérrez, [c]Ya Hua Chim, [d]Mark Field, [d]Omar Nawaytou, [c]Riaz Akhtar, [a*]Jillian Madine

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Objective: To explore the relationship of aortic medial amyloid with biochemical and micromechanical properties of the aortic wall in aneurysm patients.

Methods: Human aortic tissues removed during aneurysm surgery from tricuspid (idiopathic degenerative aneurysm, DA) and bicuspid valve (BAV) patients were subjected to oscillatory nanoindentation experiments to determine localised mechanical properties of the tissue (shear storage modulus, G´ and shear loss modulus, G˝). Collagen, elastin, matrix metalloproteinase 2 and glycosaminoglycans concentrations were determined, along with relative levels of aortic medial amyloid-related factors (medin, milk fat globule-EGF factor 8, oligomers and fibrils). Measurements were combined with clinical data and statistical analyses performed.

Results: The DA cohort can be divided based on their phenotype. One group shared similar characteristics with BAV patients, termed bicuspid like phenotype-tricuspid valve. The second group had high amyloid oligomer species present with a significantly lower G´ ($p=0.01$), indicative of reduced elastic response of the tissue, termed amyloid-rich.

Conclusions: We identified a group of DA patients with high amyloid oligomers and altered micromechanical properties of the vessel wall. We propose these findings as a cause for aneurysm formation in these patients. Amyloid is not found in BAV patients, suggesting at least two distinct mechanisms for pathogenesis.

P14: **A shark from Napoleonic wars: 3D segmentations of the organelles from the Greenland shark (*Somniosus microcephalus*) cardiac myocytes provide insights on extreme longevity**

Authors: Pierre Delaroche, Christian Pinali, Holly Shiels

University of Manchester

Abstract: The Greenland shark (*Somniosus microcephalus*) live up to 392 ± 120 years, making it the world's oldest-living vertebrate. Because cardiovascular diseases are synonymous with age in humans, we aimed to understand how the heart of this vertebrate can beat since Shakespearian times without failing. Our objective was to elucidate morphological characteristics of organelles associated with natural aging, the mitochondria and the nuclei. Heart tissue samples from the compact region of the Greenland shark ventricle were collected from a ~200 year old female Greenland shark and processed for serial block-face scanning electron microscopy according to the Ellisman protocol. Serial images were collected using Gatan 3View and analysed with IMOD. Heart tissue samples from female Greenland shark (aged 108-220 years-old) preserved in formalin were processed following immunohistochemistry procedures. Image analysis was performed using ImageJ. Approximately 1,200 mitochondria were reconstructed providing a mitochondrial volume density of 69% which is higher than that found in other polar fishes, and similar to that found in highly aerobic muscles such as billfish heater cells, which may reflect aerobic need relative to its cold environment. It can be a consequence of mitochondrial biogenesis which is known to contribute to longevity in a variety of species. Clues for mitochondrial fusion, the shape of the cardiomyocyte nuclei and the heterochromatin arrangement further support a phenotype resilient to age. In the future, our dataset will be complemented with an increased sampling size, comparisons with juvenile Greenland shark cardiac myocytes and molecular assessments investigating mitochondrial dynamics.

P15: **Prostanoid-mediated inhibition of IL-6 trans-signalling in pulmonary arterial hypertension: a role for "suppressor of cytokine 3" (SOCS3)?**

Authors: Gillian A. Durham, Jacobo Elies-Gomez, M. Talat Nasim, Timothy M. Palmer.

University of Bradford

Inflammation has been highlighted as a key factor in pulmonary arterial hypertension (PAH) development[1], in particular interleukin-6 (IL-6)[2]. IL-6 *trans*-signalling activates JAK/STAT signalling to induce transcription of pro-inflammatory and pro-angiogenic genes, enabling PAH progression, as well as the transcription of suppressor of cytokine signalling 3 (SOCS3) which limits IL-6 signalling[3]. Current PAH therapies include prostanoid drugs which induce vasodilation via stimulating intracellular cyclic adenosine monophosphate (cAMP) levels. cAMP is also an inhibitor of endothelial dysfunction via induction of SOCS3[4].

Thus, my studies are testing the hypothesis that an important mechanism by which cAMP-mobilising prostanoid drugs limit PAH is by inhibiting IL-6-mediated pulmonary inflammation and remodelling via SOCS3 inhibition of IL-6 induced JAK/STAT signalling. We have demonstrated that prostanoid drugs beraprost and treprostinil both induce SOCS3 mRNA and protein in pulmonary arterial ECs to inhibit IL-6 mediated Tyr705 phosphorylation of STAT3 by 30% ±8 ($P<0.01$) and 25% ±9 ($P<0.05$) respectively for $n=4$ experiments. We will also present data assessing the functional significance of prostanoid mediated inhibition of IL-6 signalling in pulmonary arterial ECs, and determine the EPAC1 and SOCS3 dependence of their inhibitory effects on IL-6 signalling. From these and future studies, it is anticipated that more effective strategies will emerge with which to target the IL-6/JAK/STAT signalling pathway in PAH. **References:** 1) Groth A et al. (2014) Respir Res 15: 47; 2) Jasiewicz M et al. (2014) Cytokine 76: 187-192; 3) Babon JJ et al. (2014) Semin Immunol 26: 13-19; 4) Sands WA et al. (2006) Mol Cel Biol 26: 6333-6346

P16: **PROTAC-mediated degradation of phospholamban as a novel therapeutic strategy for heart failure**

Authors: Miss Emily Kathleen Gallen, Ms Sarah Memarzadeh, Dr David France & Dr Will Fuller

University of Glasgow

Reduced SERCA activity leading to impaired Ca^{2+} storage in the sarcoplasmic reticulum (SR) of ventricular myocytes is a hallmark of heart failure, and strategies to enhance Ca^{2+} reuptake by the SR are established to improve contractility in the failing heart. In its dephosphorylated state, phospholamban (PLB) inhibits cardiac muscle sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2) which reduces the size of the SR Ca^{2+} store, and in turn decreases contractility and muscle relaxation. This research aims to disrupt the inhibitory PLB:SERCA complex through proteolysis-targeting chimera (PROTAC)-mediated degradation of PLB. PROTACs are bifunctional molecules that recruit E3 ubiquitin ligases to a target protein for their ubiquitination and subsequent degradation by the proteasome. To date, the use of PROTACs in cardiovascular disease has not been investigated. We tested three compounds with functional ligands to recruit a Von-Hippel Lindau (VHL)/Cereblon (CRBN) ligase or the molecular chaperone Hsp70 to a Halo-tagged PLB (Halo-PLB). In an engineered HEK cell line expressing Halo-PLB, PROTAC-mediated recruitment of a VHL ligase successfully degraded the protein by 60 %. Functional studies of contractility in transfected neonatal ventricular rat myocytes expressing unphosphorylatable Halo-PLB are underway. Data collected thus far supports the development of ligands to target endogenous PLB for recognition by PROTACs as a novel therapeutic strategy to promote Ca^{2+} uptake into the SR and enhance inotropy and lusitropy in ventricular myocytes.

P17: **Do the protein kinases CDK12 and CDK13 interact in the developing heart?**

Authors: Tushar K Ghosh, Qazi W Ullah, José J. Aparicio-Sánchez**,** Sarah Buxton**,** Sophie Rochette, Siobhan Loughna and J. David Brook

University of Nottingham

CDK12 and CDK13 are cyclin-dependent protein kinases that regulate cell cycle progression, transcription and splicing. In humans, only *CDK13* is associated with syndromic congenital heart disease (s-CHD). Mutations in *CDK13* result in abnormal heart and other development defects in the brain and skeletal muscles. Given the role of CDK13 in the developmental processes of heart, brain and skeletal muscle formation, this study focuses on the tissue specific expression profile of Cdk13 both in embryonic and adult mice and seeks to understand how functional deficiency of Cdk13 results in s-CHD. We have analysed *Cdk12*$^{+/-}$ and *Cdk13*$^{+/-}$ mice which are viable and do not show any obvious phenotype. We have studied the changes in the protein level of Cdk13 and the closely related Cdk12 in these heterozygous mice. Cdk12 shares 43% sequence identity with Cdk13 and they have a similar central kinase domain, suggesting the possibility of functional complementarity. In order to test this hypothesis we are generating double heterozygous mice (Cdk12$^{+/-}$ Cdk13$^{+/-}$) and we propose to analyse the changes in protein levels and study the phenotypic consequences in these heterozygous mice employing high resolution episcopic microscopy (HREM).

P18: **DHHC3-mediated palmitoylation in NCX1 trafficking and function**

Authors: Caglar Gok, Fiona Plain, Ana Costa, Will Fuller

University of Glasgow

The cardiac Na/Ca Exchangers (NCX1) is an antiporter membrane protein that regulates cytoplasmic Ca2+ by facilitating its electrogenic exchange for 3 Na+. NCX1 is implicated in the pathogenesis of heart failure and number of arrhythmias. NCX1 is palmitoylated at position 739 in its regulatory intracellular loop and loss of palmitoylation in NCX1 altered its inactivation. Despite the importance of palmitoylation on NCX1 mediated current, the palmitoylation mechanism of NCX1 still remains unclear. An enzyme family called zDHHC palmitoyl acyl-transferases (zDHHC-PATs) governs palmitoylation. Herein, we screened for the candidate zDHHC-PAT(s) interacting with NCX1 using affinity purification with an NCX1 peptide representing residues 740-756 which forms an amphipathic α-helix and is required for palmitoylation of NCX1. zDHHC 3, 7, 14, 15, 22 and 24 were shortlisted as candidate zDHHC-PATs. Strikingly, we found that only overexpression of zDHHC3 significantly enhanced the palmitoylation of NCX1. Furthermore, we probed NCX1 activity in Neonatal Rat Ventricular Myocytes (NRVM) transiently co-transfected with YFP and CFP tagged NCX1 at position 266 of the intracellular loop using Forster Resonance Energy Transfer (FRET). Overexpression of DHHC3 drastically increased FRET activity of NCX1 in NRVM cells in contrast to cells transfected with NCX1 only and NCX1 co-transfected with DHHS3; catalytically non-functional DHHC3. We, next, asked if NCX1- zDHHC3 interaction affects spatial organization of NCX1 in the cell. Overexpression of DHHC3 enhanced Golgi localization of NCX1 by increasing its palmitoylation. Currently we are studying calcium transients and contractility in NRVMs for further insights into functional aspect of zDHHC3 mediated palmitoylation.

P19: **Overload-induced skeletal muscle hypertrophy is impaired in a rat model of heart failure with preserved ejection fraction**

Authors: Ever Espino, Peter Tickle, Jack Garnham, Stuart Egginton, T. Scott Bowen

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Introduction: Heart failure with preserved ejection fraction (HFpEF) is characterized by loss of skeletal muscle mass and strength. This study aimed to further characterize the skeletal muscle phenotype in HFpEF and whether the hypertrophic response is impaired. **Methods:** Lean (n=4) and obese (n=4) diabetic Zucker fatty/Spontaneously hypertensive heart failure F1 hybrid (ZSF1) were compared at 20 weeks of age, when HFpEF develops in the obese strain. Rats underwent surgery to induce functional overload for 14 days of the extensor digitorum longus (EDL) muscle following removal of the tibialis anterior (TA). TA fibre size (histology), soleus contractile function (*in vitro* direct stimulation), and *in situ* mitochondrial respiration (high-resolution respirometry) were assessed. **Results:** Obese ZSF1 rats demonstrated an increase in body weight ($P<0.01$) and blood glucose levels by 29% and 99%, respectively ($P<0.01$). TA fibre atrophy of ~50% was observed in HFpEF compared to controls (3172±527 vs. 5667±332 um^2; $P<0.05$), with the mitochondrial respiratory coupling ratio (RCR) tending to be lower in HFpEF (6.32±0.34 vs. 9.39±1.25; $P=0.06$). Soleus twitch force was ~30% lower in HFpEF compared to controls, with peak power reduced by ~30% (0.95 vs. 1.33 W/cm^2; $P<0.05$). Functional overload increased muscle mass by 26±2% in controls but only by 19±1 % in HFpEF ($P<0.01$). **Conclusions:** Severe skeletal muscle atrophy and contractile dysfunction were present in rats with HFpEF. HFpEF was also associated with an impaired skeletal muscle hypertrophic response to mechanical overload, which may indicate an impairment in the protein synthesis signalling pathway.

P20: **TNF-α and IL-1β alter calcium handling and myofilament sensitivity in sheep ventricular myocytes**

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Pro-inflammatory cytokines such as TNF-α and IL-1β are implicated in the pathogenesis of inflammatory disease states such as sepsis. In sepsis, myocardial depression is a leading cause of death. To improve the understanding of the cellular basis we characterised the effects of TNF-α and IL-1β on intracellular calcium handing and myofilament sensitivity to calcium using a large animal model.

Sheep ventricular myocytes were used for all experiments. All procedures used accord with the Animals (Scientific Procedures) Act, UK, 1986 and Directive 2010/63/EU of the European Parliament. Intracellular calcium and contractility dynamics were measured by epi-fluorescent photometry and video sarcomere detection respectively. Cells were separately exposed to 50ng/ml TNF-α and IL-1β acutely. When required, cells were excited using field stimulation at a rate of 0.5 Hz.

Both TNF-α and IL-1β decreased SR Ca content by 27% and 41% respectively accounting for a 17% and 24% decrease of systolic Ca. Only TNF-α reduced contractility (by 20%) while IL-1β did not. Though SERCA was unaffected, the fact that both cytokines decreased the threshold for Ca waves suggests that both cytokines potentiate the ryanodine receptor, which may account for the decreased SR Ca. Findings from several experiments – including attenuation of sarcomere shortening over equivalent calcium ranges – indicate that both cytokines reduce myofilament sensitivity to calcium.

These findings considerably advance our understanding of the effects of TNF-α and IL-1β on cardiac cellular function and provide additional substrates that can account for aspects of myocardial dysfunction in inflammatory diseases such as sepsis.

P21: **Remodelling of the ryanodine receptor cluster pattern within an acute model of right ventricular heart failure**

Authors: Miriam E. Hurley, Thomas M.D. Sheard, Ruth Norman, Eleftheria Pervolaraki, Derek Steele, Ed White and Izzy Jayasinghe

University of Leeds

Within a cardiomyocyte, contractile proteins are activated by calcium. Calcium is released in bursts from the ryanodine receptor (RyR). Clusters of the RyR are located upon the cell's internal calcium store, the sarcoplasmic reticulum, to form a structural unit known as a couplon. In right ventricular heart failure (RV HF) there is apparent structural remodelling of the couplon, in particular RyR cluster organisation. At a local level, the functional implication of this structural remodelling is not fully understood.

Isolated right ventricular cardiomyocytes were obtained from adult male Wistar rats. Rodents were either injected with monocrotaline (60 mg/kg) to induce pulmonary artery hypertension which led to compensated RV HF (MCT-RV) or with saline (Ctrl-RV) as previously established. Cardiomyocytes from MCT-RV and Ctrl-RV were imaged using the new super-resolution microscopy method, DNA-PAINT, exploiting its ability to resolve single RyR *in situ*.

Remodelling across the right ventricle was heterogeneous within this acute model of RV HF. Fragmentation of the near-surface RyR cluster pattern was observed within MCT-RV cardiomyocytes. This was evident by a reduction in the average peripheral RyR cluster size in MCT-RV, compared to Ctrl-RV.

Using a correlative imaging protocol, we have examined the functional implication of an altered RyR pattern upon a cardiomyocyte's local calcium signalling properties. With this, we observe an increased frequency of spontaneous calcium release in the local regions which exhibit a fragmented RyR pattern.

P22: **Expression of the voltage-gated sodium channel isoforms $Na_v1.5$ and $Na_v1.8$ in human tissue are altered with patient's increasing age.**

Authors: E. Isaac, M. Chaudhry, M. Loubani and S.A.Jones

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Background: Sodium channels are implicit in the appropriate depolarisation of cardiomyocytes, and changes in isoform expression may lead to potentially arrhythmogenic behaviour. Identifying sodium channel expression isoforms $Na_v1.5$ and $Na_v1.8$ changes in protein expression with increasing age may shed light on a key physiological process underpinning atrial arrhythmias.

Aim: To Investigate the right atrial tissue derived from patients of increasing age for changes in the protein expression of voltage-gated sodium channel isoforms $Na_v1.5$ and $Na_v1.8$.

Methods: Right atrial appendage tissue from patients undergoing routine cardiac surgery (IRAS Approved ethics). Protein expression of $Na_v1.5$ and $Na_v1.8$ was investigated using western blot (WB) and immunocytochemistry (I) with confocal microscopy. The primary antibodies $Na_v1.5$ and $Na_v1.8$ (Alomone, Israel) were used at the dilutions of 1:500 (WB) and 1:100 (I), and the rabbit secondary antibody 1:1000 (conjugated to either HRP or fluorescein; ThermoFisher, UK). Confocal images and band densities were quantified using Image J software. Data mean ± SEM, Student's t-test $p<0.05$.

Results: Using both techniques we identified a significant decrease in the protein expression of the $Na_v1.5$ isoform, accompanied by an increase in $Na_v1.8$ isoform in human tissue derived from patients of increasing age.

Conclusion: Old age is associated with a reduced expression of $Na_v1.5$ and increased expression of $Na_v1.8$. This inverse relationship of sodium channel isoform expression at an older age may underpin the development of arrythmiogenic action potentials and potential rise in atrial arrhythmia experienced by the elderly population.

P23: **Cardiotoxic effects of phenanthrene in zebrafish myocytes**

Authors: Shiva Nag Kompella[1], Jules Hancox[2], Chris Dempsey[2], Fabien Brette[3], Holly Shiels[1]

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Polyaromatic hydrocarbons (PAH) which form an important component of crude oil are shown to have carcinogenic and cardiotoxic effects. Recent studies show a rise in concentration of phenanthrene, a three ring PAH, due to oil weathering and exhibited direct inhibitory effects on Ether-à-go-go (erg) ion channel of various fish cardiomyocytes which can lead to Long QT syndrome (LQTS). Phenanthrene also forms a significant component of air pollution. Our study aimed to understand the cardiotoxic effects of phenanthrene inisolated zebrafish cardiomyocytes. Action potential (AP) of zebrafish ventricular cardiomyocytes exhibit close resemblance to that of human cardiomyocytes along with a 63% sequence homology in erg channel, making an important model system. Manual patch clamp recording of zebrafish ventricular cardiomyocytes reveal a dose-dependent inhibition of erg channel. Interestingly, phenanthrene causes a significant reduction in AP duration (APD) at 50% (APD50) which primarily represent the activity of L-type calcium channel. Our data reveal inhibition of erg channel along with possible inhibitory effect of L-type calcium channel by phenanthrene indicating a strong cardiotoxic effect on zebrafish ventricular cardiomyocytes.

P24: **Investigating palmitoylation of cardiac myosin binding protein-C in cardiac health and disease**

Authors: Alice Main, George Baillie, Will Fuller

University of Glasgow

Cardiac myosin binding protein-C (cMyBP-C), a sarcomere thick filament protein, is partly responsible for phosphorylation-dependent control of cardiomyocyte contractility. Although known for its role in hypertrophic cardiomyopathy, recent evidence suggests cMyBP-C may be involved in the pathophysiology of other cardiac diseases including myocardial infarction and heart failure, where PKA phosphorylation is reduced and cardiac contractility is compromised. As cMyBP-C is a non-enzymatic protein, understanding how it functions via protein-protein interactions and post-translational modifications is essential to discovering its role in cardiovascular disease. Palmitoylation is the addition of a 16-carbon fatty acid to cysteine residues, and regulates the location, and stability of a protein. Several studies have shown the how palmitoylation plays a crucial role in the function of a number of cardiac substrates. Preliminary data from our group suggests cMyBP-C is palmitoylated in ventricular myocytes. Resin-assisted capture of acylated proteins suggests the developmental stage and culture of cardiomyocytes influences the extent of MyBP-C palmitoylation. Additionally, cardiomyocytes isolated from a model of heart failure (8-week post-myocardial infarction New Zealand White Rabbits) show reduced cMyBP-C palmitoylation compared to control. Finally, we have established a novel technique, which allows palmitoylation site identification using peptide array, with sites then confirmed using site-directed mutagenesis. Future analysis of the effect of cMyBP-C palmitoylation in relation to phosphorylation and overall cardiomyocyte contractility will reveal the role this modification plays in cMyBP-C function in cardiac health and disease.

P25: **Evaluation of toxic effects of amyloid beta 1-42 peptide on Blood Brain Barrier**

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The blood brain barrier (BBB) is the boundary layer of specialized endothelial cells that separates blood from brain interstitial fluids. BBB is crucial for maintaining the homeostasis of the brain microenvironment and prevention of entry of toxic substances into the central nervous system (CNS). The BBB cells consists of capillary endothelial cells, pericytes encircling the endothelium and surrounding astrocytes, which all play key roles in the physiology and pathology of the CNS. BBB dysfunction is implicated in various neurodegenerative diseases, including Alzheimer's disease (AD). Amyloid β (Aβ) peptide is the major component of amyloid plaques, the primary toxicant in AD brains, and has a central role in AD pathology. The toxic effects of $A\beta_{1-42}$ peptide fragment have been widely investigated on neurons. However, toxicity of $A\beta_{1-42}$ on BBB cells (e.g. endothelial cells, astrocytes and pericytes) is poorly understood. Herein, we investigated effects of this peptide on human primary BBB cells. We observed that pericytes were affected by $A\beta_{1-42}$ exposure after 24h treatment (0.1, 0.5 and 1 μM), whereas viability of astrocytes and endothelial cells was not significantly altered by the presence of the peptide at this period. This study provides a novel insight into the effects of $A\beta_{1-42}$ on primary BBB cells, in particular the potential role of pericytes in AD.

P26: **Characterisation of novel platelet biomarkers using Arginine Methylation.**

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Introduction: One of over 200 known post-translational modifications which are known to occur in mammalian cells, Arginine Methylation (ArgMe) is catalysed by Protein Arginine Methyltransferases (PRMTs). These experiments have used Furamidine, a novel PRMT1 inhibitor which has been found to reduce the ArgMe of platelet proteins, and functionally inhibit platelet aggregation. **Results:** Bioinformatic analysis of proteomic data showed that out of 4058 platelet proteins, 260 (6.4%) are modified by arginine methylation and were enriched in the gene ontology term 'Platelet Aggregation'. Included in these proteins was VASP which was found to be methylated at R67 and R298. VASP was found to be arginine methylated in donor samples, tested using arginine methylation antibodies α-RGG and α-R4, in western blot and immunoprecipitation experiments. Incubation with the novel PRMT1 inhibitor furamidine functionally inhibited platelets in *ex vivo* analysis of platelet aggregation. The IC50 of furamidine was found to be 13.46μM (Thrombin) and 3.17μM (Collagen). Incubation of platelets with furamidine showed a decrease in arginine methylated proteins, corresponding with aggregation data. **Conclusion:** Through this study, the dysfunction of platelets has been linked to ArgMe through inhibition of PRMT1 by furamidine. As the dysfunction of platelets *in vivo* has been linked to the onset and progression of cardiovascular disease, further research into the arginine methylome of platelets and the possibility of using furamidine as a possible, novel antiplatelet treatment may provide a more effective mechanism for treating the 7 million people who are fighting heart disease in the UK every day.

P27: **Three-Dimensional Reconstruction of the Mouse Atrioventricular Node Cells using Serial Block-Face Scanning Electron Microscope.**

Authors: Pia Morales, Dr Ashraff Kitmitto, Prof Mark Boyett, Dr Sunil Logantha

University of Manchester

The atrioventricular node (AVN) is part of the sole conduction pathway connecting the atria and the ventricles and it is critical to normal heart functioning. It can be affected by relatively common arrhythmias. When first-degree atrioventricular block affects heart failure patients, it can lead to death. The main limitation in the progress towards developing better treatment strategies is that the structure-function relationship of the AVN remains enigmatic. To elucidate that, it is crucial to understand its cell volumetric configuration at the ultrastructural level. Data about the microarchitecture of the AVN are only two-dimensional micrographs. Therefore, this project has generated high-resolution tri-dimensional reconstruction of the mouse AVN myocytes. Mice AVN samples of 1x1 mm have been taken, and images obtained using serial block-face scanning electron microscopy (SBF-SEM). EM images have been analysed and AVN myocytes manually segmented using IMOD. Data from the AVN has been compared with those from atria samples. AVN myocytes have shown to have significantly small cell volume (p-value=0.007) and cell-surface area (p-value=0.007) compare with atrial myocytes. The same is true for nuclei (volume p-value=0.003 and surface area p-value=0.002). The AVN tissue samples have also shown more cell heterogeneity. In conclusion, the mouse AVN myocytes are distinct from those found in the atrial samples. We expect, this project will further bridge our understanding of the AVN and thus progress to clinical translation.

P28: **How does time of day affect gene expression in the neuronal controllers of vascular tone, sympathetic preganglionic neurones?**

Authors: C. Nathan, J. Aspden, S. Deuchars, J. Deuchars

University of Leeds

Blood pressure exhibits diurnal rhythms, absence of which is correlated to an increased risk of developing cardiovascular diseases. Blood pressure is to a large part controlled by sympathetic nervous system activity, which we have shown exhibits diurnal activity. Since sympathetic preganglionic neurons (SPNs) are the final common pathway for central nervous system influence on blood pressure, this project aims to determine if SPN function could be regulated by diurnal expression of genes. The diurnal expression of genes encoding proteins involved in determining neuronal activity were investigated in RNA extracted from micro-punches that included the location of the majority of SPNs, the intermediolateral cell column (IML) at 7:30 AM and 7:30 PM of C57/Bl6 mice (N= 10). qPCR revealed mRNA levels of Bmal1 and Per2 varied with time of day in the IML; Bmal1 mRNA was higher in the morning while Per2 mRNA was higher during the evening. Diurnal rhythm of Bmal1 and Per2 protein levels in SPNs were then examined using immunofluorescence. Bmal1 and Per2 protein levels within the SPN nucleus vary with time of day with Bmal1 levels being higher in the morning (N=5 animals, n=15 sections). To investigate a broader sample of genes, RNAseq was performed on micropunches obtained as above. Diurnal rhythm of expression was observed in potassium channel subunits (e.g. Potassium voltage-gated channel, Shaw-related subfamily, member 3) and glutamate subunits (e.g. Glutamate receptor, ionotropic, N-methyl D-aspartate-associated protein 1) consistent with increased neuronal activity. Analysis of RNAseq is being undertaken to further examine the expression of which genes vary with time of day.

P29: **Exploring Cardiac Caveolae: Protein quantification and arrangement**

Authors: Ruth Norman, Richard Bennett, Thomas Sheard, Ben Nichols, John Colyer, Izzy Jayasinghe, Sarah Calaghan

University of Leeds

Caveolae, small ~80 nm membrane invaginations, regulate numerous membrane receptors and signalling pathways within cardiac myocytes. However much of the current knowledge of caveolae is based on experimental evidence from cell lines which do not contain the muscle specific caveolar proteins (caveolin-3 and cavin-4). There we examine the populations and protein composition of cardiac caveolae using quantitative protein analysis, protein coat isolation and super-resolution imaging. Cardiac myocytes, isolated from male Wistar rats, were attached to coverslips from super-resolution imaging. Paraformaldehyde-fixed myocytes were labelled with caveolin-3 and cavin-1/4 antibodies, 10X Expansion-Microscopy was then performed to achieve ~25nm resolution. In cell homogenates caveolar protein abundance was quantified using stable isotope dilution mass spectrometry with custom-made calibration standards. Hela cells and cardiac myocytes were incubated with the cross-linker dithiobis(succinimidylpropionate)(DSP) for isolation of the caveolar coat complex (CCC) on a sucrose velocity gradient. Caveolin-3 has the highest expression within myocytes followed by cavin-1/4 (~60% of caveolin-3). Caveolin-1/2 and cavin-1/2 are detected at lower levels (<20% of caveolin-3). The CCC isolated from Hela cells was ~80S in size and contained the main ubiquitous isoforms (caveoline-1 and cavin-1). In myocytes, the ~80S complex contained only caveolin-1 and cavin-1/4. Despite being the predominant isoform caveolin-3 was absent from these complexes in the cardiac cell. Indeed, caveolin-3 migration in the sucrose gradient did not change with DSP, suggesting that caveolin-3 does not integrate into the CCC in muscle cells as caveolin-1 does in non-muscle cells. Using Expansion-Microscopy, caveolin-3 and cavin-1 can clearly be resolved within a single caveolae with high levels of co-localisation. Taken together these data highlight distinct differences in caveolae between myocytes and non-muscle cells. Understanding the protein composition of the different caveolae is essential in understanding the multiple functions that caveolae perform.

P30: **Regional variations of biomechanical properties of the ovine aorta at the macro- and micro- scale correlation with in collagen, elastin and glycosominoglycan levels**

Authors: Panpho, P.[1], Field, M.[2], Madine, J.[3], Akhtar, R.[1]

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Aortic diseases are a significant cardiovascular health problem and occurs in different ways across the vascular tree. Investigation of the mechanical properties of the aorta is important for better understanding of aortic diseases. While there have been previous studies examining the alterations in the macroscopic biomechanical behaviour and how they correlate well with regional microstructural changes, little is known about how these properties vary across its entire length. Our study presents maps the biomechanical properties (at the macro-scale via tensile testing and micro- scale via oscillatory nanoindentation and biochemical properties (Collagen, Elastin and GAG) along its entire length of the ovine aorta. Three ovine aortas were used for nanoindentation testing. For nanoindentation, the entire aorta was split into nine transverse sections. The aorta was divided into sections separated by 2 cm in length from the aortic root to the celiac artery region. Each of these sections were used to create three 5-millimeter circle biopsy punches for nanoindentation (a total of 81 biopsies). 16 oscillatory indentations were applied to the surface of the intimal and adventitia layer. Subsequently, the same samples were used to determine elastin, collagen and glycosaminoglycan (GAG) levels using established biochemical assays. Overall, our study found that there is a significant increase in macroscopic and micromechanical properties from the ascending aorta to the abdominal aorta. There was a significant correlation between an increase in G' ($P<0.0001$) and collagen ($P=0.001$) with distance from the aortic root whilst elastin ($P=0.001$) and GAG ($P=0.01$) levels were significantly decreased.

P31: **Determining how mutations in β-cardiac myosin cause hypertrophic cardiomyopathy.**

Authors: F Parker, M Wolny, RE Hughes, and M Peckham

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Hypertrophic cardiomyopathy (HCM) is a cardiovascular disorder that affects 1:500 people and is thought to be the lead cause of sudden adult death in young people and athletes. Mutations in the motor protein β-cardiac myosin heavy chain (MHC) are responsible for around 35 % of cases and a fifth of these mutations are found in the S2-domain. This study investigates point mutations at the proximal S2 region, close to the head/tail junction, where the coiled-coil tail is predicted to separate to allow flexibility during contraction and to aid the interaction of the head with the tail when the MHC is in the super-relaxed, switched off state. Pathogenic mutations were introduced into a S2 peptide (126aa) at 3 amino acid residues; R858 →C, G, H or P; R869→C, G or H and R870 →C, H or L. Circular dichroism showed 6 out of 10 mutations were less helical and thermally stable than WT. These 6 mutations were introduced into GFP-tagged full-length MHC and expressed in cultured myotubes. All the mutant MHCs, except R858P, incorporated normally into sarcomeres. So far, our studies suggest pathogenic mutations destabilise the coil at the head-tail junction, but mostly do not affect incorporation of myosin into filaments. Destabilisation of the S2-domain would be expected to weaken force transmission from the motor to the tail during contraction, and may affect the ability of MHC to form the super-relaxed switched off state.

P32: **Investigating the beneficial effects of voluntary exercise in rats with pulmonary artery hypertension**

Authors: Eleftheria Pervolaraki, Ed White, Al P. Benson

University of Leeds

Voluntary wheel running can delay the onset of right ventricular failure (RVF) in rats with pulmonary artery hypertension (PAH) induced by monocrotaline (MCT) (Natali et al, 2015 Am.J.Physiol.309:H421-4). Although the mechanisms associated with this benefit are unknown, electrical, Ca2+-handling and structural remodeling are associated with cardiac dysfunction MCT rats.

Male Wistar rats (185g) were injected with 60mg/kg MCT to induce PAH and RVF, or an equivalent volume of saline as controls (CON). Animals were randomly assigned free access to a running wheel (exercise; ex) or not (sedentary; sed). SedMCT animals were sacrificed upon external signs of heart failure; CON and exMCT animals were sacrificed on matched days to sedMCT animals, unless heart failure signs developed earlier in exMCT animals.

Isolated hearts were Langendorff perfused and epicardial membrane potential optically mapped using RH237, Ca2+ transients were simultaneously measured using Rhod-2. Signals were recorded with external pacing at 5 Hz. Following optical mapping, hearts were preserved in 5% paraformaldehyde by immersion and imaged with diffusion tensor MRI using a Bruker 9.4 T system and a diffusion weighted spin-echo protocol to reveal myocardial fiber structure at a resolution of 200 μm isotropic.

Preliminary studies confirm voluntary exercise prolongs survival, no exMCT animals developed heart failure signs on the day of such signs in sedMCT animals (21±0.26 days, n=6). MCT treatment significantly increased RV action potential duration (APD80, MCT 71.6±7.7ms; CON 42.3±5.4 ms, $P<0.05$ n=5) and fibre disarray (R2, MCT 0.84±0.04; CON 0.47±0.04, $P< 0.001$, n=3-8). No exMCT values were above the median MCT value suggesting a trend for attenuation of these effects by exercise. Supported by the BHF.

P33: **LET'S TAKE A *CELL*-FIE! Studying the ultrastructure of the sinoatrial node using serial block face scanning electron microscopy.**

Authors: Kevin Shaji, Professor Mark Boyett, Dr. Ashraf Kitmitto and Dr. Sunil Jit Logantha
University of Manchester

The sinoatrial node (SAN) comprises a bundle of specialised cells within the heart that possess the fastest rate of spontaneous automatic impulse generation. Therefore, it is referred to as the primary pacemaker of the heart. Although our understanding has immensely improved, much regarding the node still remains to be fully elucidated. Particularly, with regards to the ultrastructure of the SAN as electron microscopy investigation until now has been limited to two-dimensional analysis. Therefore, we aim to provide an insight into the three-dimensional microscopic architecture and organisation of the SAN, by employing a novel approach known as serial block face scanning electron microscopy (SBF-SEM).

The mouse heart was dissected, and 1x1 mm biopsies of the central SAN region surrounding the SAN artery were acquired. Following established protocols, these samples underwent appropriate preparation for electron microscopy imaging. Ultra-high-resolution images of the mouse SAN were captured via SBF-SEM. The images were visualised, manually segmented, and the *in-situ* three-dimensional nodal ultrastructure was reconstructed using IMOD software. Our observations reveal that the SAN is composed of a heterogeneous mixture of cells: cardiomyocytes, fibroblasts, macrophages, monocytes, and telocytes embedded within an abundance of connective tissue and nerve fibres. SAN cardiomyocytes have a characteristic spindle or spider shape and make contact with each other at intercalated discs. Furthermore, in comparison with right atrial myocytes, SAN cells were found to be significantly different in terms of volume (atrial: $6.773643333 \times 10^9 \pm 1.676345137 \times 10^9$ μm^3 vs. SAN: $1.266551 \times 10^9 \pm 2.8990335 \times 10^8$ μm^3; $p<0.05$; n=3/3) and surface area (atrial: 4606127±868350 μm^2 vs. SAN: 1683453±319489 μm^2; $p<0.05$; n=3/3).

P34: **Expansion microscopy reveals dyadic ryanodine receptor nanodomain phosphorylation changes in monocrotaline-induced right ventricular heart failure**

Authors: Thomas M. D. Sheard, Miriam E. Hurley, John Colyer, Ed White, Ruth Norman, Eleftheria Pervolaraki, Kaarjel K. Narayanasamy, Yufeng Hou, Hannah Kirton, Zhaokang Yang, Liam Hunter, Jung-uk Shim, Alexander H. Clowsley, Andrew J. Smith, David Baddeley, Christian Soeller, Michael A. Colman, and Izzy Jayasinghe
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The ryanodine receptor (RyR) calcium channel is tightly clustered into signalling nanodomains, which comprise the calcium release units of cardiomyocytes. Super-resolution microscopy has previously been used to visualise RyR clusters at the individual protein level, near the cell surface. The structure of nanodomains located deeper, for instance dyadic clusters associated with t-tubules, has remained unresolved due to limited imaging depths and axial resolution of these techniques.

A series of enhancements made to expansion microscopy, a novel super-resolution technique based on swellable hydrogels, allowed individual RyRs to be resolved within surface clusters, as well as interior nanodomains, in isolated cardiomyocytes. With ~ 15 nm we localised the position of RyRs, as well as the positions of RyRs phosphorylated at the residue Ser2808.

Using the monocrotaline (MCT) model of right ventricular (RV) heart failure we observed disturbances to the interior RyR arrays in MCT-RV, coinciding with t-tubule remodelling. RyR hyperphosphorylation in MCT-RV appeared in a gradient from the edge of the nanodomain towards the centre, which was not seen in control. This spatial profile contrasted with that observed in cells stimulated with the β-adrenergic agonist isoproterenol, which mimics acute, physiological hyperphosphorylation. Simulations of RyR arrays based on the experimentally-determined channel positions and phosphorylation signatures showed how nanoscale dispersal of the RyRs during pathology diminishes its intrinsic likelihood to ignite a calcium signal. It also revealed that the natural topography of RyR phosphorylation could offset potential heterogeneity in nanodomain excitability, which may arise from RyR re-organisation.

P35: **Postnatal development of t-tubules and alterations in triggered calcium release in the sheep atria**

Authors: Charlotte E. R. Smith, David A. Eisner, Andrew W. Trafford and Katharine M. Dibb

University of Manchester

Transverse (t)-tubules are membrane invaginations that facilitate synchronous calcium release and cardiac contraction. T-tubules are absent at birth in the ventricles of small mammals, where they develop postnatally with subsequent changes in calcium handling. Nothing is currently known about the development of t-tubules and changes in calcium release in the atria.

Left atrial cells were isolated from 1 week, 1 month, 3 months and adult sheep. T-tubule density was assessed using di-4-ANEPPS with spatial calcium release assessed in Fluo-3 loaded cells under perforated patch control.

T-tubule density increased during development up to 3 months where density was unchanged vs adult ($p<0.001$). As t-tubule density increased postnatally, there were improvements in the uniformity of calcium release. Whilst calcium transient amplitude was reduced in the cell centre vs surface in 1 week animals, no surface vs centre differences were observed in 3 months and adult ($p<0.01$). Compared to the ventricle, calcium release in the atria is patchy and occurs in discrete locations on the cell surface and in the cell centre – likely on t-tubules. In neonates where t-tubule density is less, the amplitude of calcium release at discrete surface sites was 47.2±25.4% greater than sites in the centre ($p<0.001$).

T-tubules are present at birth in the sheep atria and develop until ~3 months of age. Our data suggests that reduced t-tubule density results in reduced central calcium release in neonates that is compensated for by enhanced surface activity. The synchronicity of calcium release is improved in later life, coinciding with t-tubule maturation.

P36: **Gene therapy for cardiac conduction system dysfunction in heart failure**

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Introduction: Heart failure (HF) is characterised by generalised dysfunction of the cardiac conduction system (CCS). Ion channel and structural remodelling in the CCS have been demonstrated in animal models of cardiovascular disease. Given the documented roles for microRNAs (miRs) in CCS molecular remodelling, we aim to develop a tissue specific method of modulating miR expression using miR sponge transgenes. **Methods**: New Zealand rabbits were used to investigate Purkinje fibre (PF) ultrastructure and remodelling. HF was induced via pressure and volume overload as previously described. Free running PFs were serially sectioned and imaged via serial block face scanning electron microscopy (SBF-SEM). To target miR sponge transgene expression to the CCS, a GFP reporter gene was placed under control of the KCNE1 promoter, a potassium channel subunit expressed throughout the CCS, or the HCN4 promoter, a key pacemaker ion channel, to target the sinus node. **Results**: PCs were uninucleated and spindle shaped with an irregular membrane, and had an average length of 108µm, diameter 15µm, and volume 7965μm^3 (n=9). Gap junctions were abundant and distributed along the lateral surface of cells, and there was a trend towards decreased expression in HF ($p=0.0526$, n=3 cells analysed per group). Hypertrophy and nuclear membrane breakdown were evident in HF PCs, the latter facilitating mitochondrial entry. GFP expression from the KCNE1 and HCN4 promoters was low in NRCMs, but was elevated in isolated SAN-like Shox2 cells, suggesting a degree of tissue specific promoter activity. **Conclusions**: SBF-SEM revealed ultrastructure of free running PFs *in situ*, and uncovered novel structural changes in HF that are likely to be pro-arrhythmic. Preliminary results suggest that the HCN4 and KCNE1 promoters may be capable of driving CCS specific transgene expression.

P37: **Effect of a Cardiotoxic Pollutant-Phenanthrene on the Cardiac Function of Brown Trout (*Salmo trutta*)**

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Phenanthrene (Phe) is a three-ringed polyaromatic hydrocarbon which is formed from incomplete combustion of hydrocarbons and is also a component of crude oil. Previous studies have shown Phe to be cardiotoxic to marine fishes. This study investigated the cardiotoxic effect of Phe across multiple levels of cardiac organization in the brown trout, a sentinel freshwater species. Using Langendorff heart perfusion and contact electrodes we found a significant prolongation of both the QT interval and the monophasic action potential (MAP) duration following ascending doses of Phe. Phe decreased the force of contraction in isolated ventricular and atria muscle strip preparations across a force-frequency trial. This suggests that Phe is able to reduce ventricular and atria contracting force by altering cellular calcium cycling. This finding was supported by a reduction in the cellular calcium transient following Phe exposure in isolated ventricular myocytes. Single cell voltage clamp revealed a Phe-dependent reduction in the L-type Ca^{2+} current (I_{Ca}) which accounts, at least in part, for the reduced Ca^{2+} transient and force. The prolongation of MAPD and QT interval suggest that Phe could also play a role in the inhibition of repolarizing K^+ currents which was confirmed by a reduction in the delayed rectifier K^+ current (IK_r) following Phe exposure. Together our data indicate that Phe is cardiotoxic to freshwater salmonids and operates via similar mechanisms to those identified for marine teleosts.

P38: **Dyadic ultrastructure underlies spatial differences in atrial and ventricular calcium handling**

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University of Manchester

Calcium (Ca^{2+})-induced Ca^{2+} release drives cardiac myocyte contraction. Ca^{2+} release from the sarcoplasmic reticulum is determined by the availability and open probability of ryanodine receptors (RyR), which arrange into clusters. Computer modelling suggests that distance between clusters and cluster heterogeneity affects RyR Ca^{2+} release properties. Little is known about RyR cluster ultrastructure in large mammalian cardiac myocytes, and how this relates to important inter-chamber differences in the Ca^{2+} transient. The aim of this study was to determine if the spatiotemporal differences in Ca^{2+} release between the atria and ventricle are associated with differing RyR cluster geometry.

Left atrial and ventricular myocytes were isolated from healthy sheep and immunocytologically labelled for RyR. Using photo-switchable fluorescent dyes, dye-molecule switching events were acquired over 10,000 frames using the Nikon super resolution N-STORM 4.0 setup. Quantitative analysis of RyR was performed by western blot.

In both the atria and ventricle, RyRs were predominantly arranged along the Z-lines with few longitudinal projections. RyR clusters were both larger by 49.0±24.3% ($P<0.05$; n=27-35 cells) and closer together in the atria, with edge-to-edge distances decreased by 49.9±4.2% ($P<0.0001$; n=27-35 cells) versus RyR clusters in the ventricle. Western blot analysis revealed no difference in RyR expression between chambers ($P=0.49$; n=8 animals).

To summarise, the use of super resolution microscopy has shown for the first time inter-chamber differences in RyR distribution. This may account for the spatiotemporal differences in triggered Ca^{2+} release seen between the atria and ventricle. Future experiments will further characterise the spatial properties of essential Ca^{2+} proteins.

P39: **Circadian disruption caused intrinsic sinus node arrhythmias**

Authors: Yanwen Wang, Emma Weston, Rebecca Northeast, Hugh Piggins, Mark Boyett. University of Manchester

Circadian disruption has become an increasing problem in modern life. Shift worker, frequent time-zone travellers and patients with sleep disorders are all affected by different degrees of circadian disruption. These people are more prone to sinus node brady-/tachy-arrhythmias, impaired heart rate variability, sudden cardiac death. However, how circadian disruption induces sinus node dysfunction remains unexplored. In this study, I would like to explore the mechanisms behind the circadian disruption and the pacemaker of the heart, sinus node. Firstly, circadian disruption were mimicked by chronic ''jet-lag'' protocol, which consists of 3 days in 0700 lights on (Zeitgeber time (ZT) 0) 1900 lights off (ZT 12) cycle (GMT+0), then 3 days in 2300 lights on 1100 lights off cycle (GMT+8), this 6-day cycle repeat for 30 days. The occurrence (Day 0, 1/20; Day 30, 7/20) and duration (Day 0, 2.7234 s; Day 30, 11.168 s (n = 20)) of ectopic heart beats progressively increased during the 30 days circadian disruption protocol. Impaired heart rate variability with prolonged RR interval (circadian disruption, 0.1343±0.0023 ms, 0.1286±0.0026 ms, n=20, p=0.0418) were observed in the anaesthetised chronic circadian disruption mice. In the de-nervated isolated sinus node tissues, the ectopic beats with prolonged and shortened beat-to-beat intervals were observed in circadian disruption group while as regular beating rate in the control group. The chronic circadian disruption sinus node showed dampened and shifted beating rate throughout 24 hour comparing with the control group. 2 mM Cs^{+} was applied to block the pacemaker channels, the delta beating rate in the chronic circadian disruption showed shifted and dampened rhythm comparing with control sinus node. In chronic circadian disruption sinus node, the delta beating rate showed circadian rhythm with peak at ZT 6 (peak at ZT 12 in control) and trough at ZT 18 (trough at ZT 0 in control). The pacemaker current, funny current, I_f was measured in the isolated sinus node myocytes at 6 time points over 24 hours in chronic circadian disruption mice and control mice. The I_f density of chronic circadian disruption showed shifted and dampened rhythms over 24 hours comparing with control ones. Moreover, the mRNA expression of circadian genes, *BMAL1* and *CLOCK*, pacemaker channel genes, *HCN1* and *HCN4* were measured at 6 time points over 24 hours in chronic circadian disruption mice and control mice. In the chronic circadian disruption, the circadian genes were both shifted with altered mRNA expression levels, and both pacemaker channel genes were dampened and shifted comparing with control group. To summarise, chronic circadian disruption causes the disrupted rhythms of circadian genes, which transcriptionally alter the pacemaker ion channel HCN4, and the corresponding intrinsic heart rate in the sinus node.

P40: **The role of BMAL1 in the rhythmic circadian variation in the sinus node**

Authors: Emma Weston, Cali Anderson, Matthew Smith, Claire Wilson, Yanwen Wang, Mark Boyett
University of Manchester

Many biological processes show a 24-hour oscillation; this variation is controlled by circadian clocks. At a molecular level circadian clocks are self-sustaining transcription/translation loops. They are regulated by circadian genes BMAL1:CLOCK binding to E-box domains. In humans, the resting heart rate shows circadian variation. This variation was previously thought to be entirely due to autonomic tone, however we have previously shown that rhythmic expression of HCN4 also plays a role. In this study, we aimed to investigate the role of BMAL1 in regulating the circadian rhythms of the sinus node and the corresponding heart rate. Circadian rhythms *in vitro* were examined in C2C12 cells. Circadian clock genes were re-synchronised by 50% FBS serum shock protocol. Changes in key circadian genes, including *BMAL1*, *CLOCK*, *Per1*, *Per2*, *Cry1*, *Cry2* were compared between serum-shock treated cells and non-treated cells every 6 hours. *BMAL1* was knocked down by siRNA in C2C12 cells and changes in circadian genes were measured and compared with the non-treated group. Circadian rhythms in the sinus node tissue were then examined. The sinus node was dissected and cultured for 3 days, the clock genes re-synchronised by serum shock protocol and the beating rates measured and compared with the non-treated group. BMAL1 in the sinus node was knocked down using siRNA, and the beating rate was measured and compared with the untreated group. Overall, our findings suggested BMAL1 is important to maintain the rhythmic circadian variation in the sinus node.

P41: **Exploring the role of cardiac microstructure and its variability in ventricular arrhythmogenesis**

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Objective: Propagation of cardiac electrical excitations is influenced by tissue microstructure, although quantitative characterisation of this relationship remains a significant research challenge. Computational modelling of cardiac electrophysiology which incorporates both dynamic electrical activity and myocardial structure offers a viable method of studying the influence of microstructure and its variability on complex excitation patterns. **Methods:** The role of tissue microstructure (cardiomyocyte and sheetlet orientations) on normal and arrhythmic excitation patterns at the organ scale was investigated using five healthy rat ventricle reconstructions, obtained at 100 μm isotropic resolution from diffusion tensor MRI (DTI). The Fenton-Karma 3 variable action potential (AP) model was modified to reproduce the rat AP duration (APD) and its restitution. Re-entrant scroll waves were simulated in the five anatomical models at prescribed locations for three different microstructure scenarios: (i) isotropic (no microstructure); (ii) anisotropic (myocyte but no sheetlet microstructure); and (iii) orthotropic (myocyte and sheetlet microstructure). **Results:** Inclusion of DTI-based microstructure increased the number and length of scroll wave filaments. The extent to which microstructure modulated arrhythmia dynamics differed between the five reconstructions, highlighting the important and under-appreciated role of structural variability. **Conclusion:** This study shows that microstructure variability influences arrhythmia dynamics including specific properties of scroll wave filaments.

P42: **Dynamic-clamping human and rabbit atrial calcium current: narrowing I_{CaL} window abolishes early afterdepolarisations.**

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Background: Atrial early-afterdepolarisations (EADs) may contribute to atrial fibrillation (AF), perhaps involving reactivation of L-type Ca^{2+} current (I_{CaL}) in its window-region voltage range. ***Aims:*** Validate the dynamic-clamp technique for modifying I_{CaL} contribution to atrial action potential (AP) waveform; investigate effects of widening window-I_{CaL} on EAD-propensity; test whether EADs from increased I_{CaL} and AP duration are supressed by narrowing window-I_{CaL}.

Methods and Results: I_{CaL} and APs were recorded from rabbit and human atrial myocytes by whole-cell-patch-clamp. During AP-recording, I_{CaL} was inhibited (3 μM nifedipine) and replaced by a dynamic-clamp model-current, $I_{CaL,D\text{-}C}$ (tuned to native I_{CaL} characteristics), computed in real-time (every 50 μs) based on myocyte membrane potential. $I_{CaL,D\text{-}C}$-injection restored the nifedipine-suppressed AP plateau. Widening window-$I_{CaL,D\text{-}C}$, symmetrically by step-wise simultaneous equal shifts of half-voltages ($V_{0.5}$) of $I_{CaL,D\text{-}C}$ activation (negatively) and inactivation (positively), generated EADs (single, multiple, or preceding repolarisation-failure) in a window-width-dependent manner, and AP-alternans. A stronger EAD-generating effect resulted from independently shifting activation $V_{0.5}$ (asymmetrical-widening) than inactivation $V_{0.5}$; e.g. a 15-mV activation-shift produced EADs in 9/17 (53%) human atrial myocytes *versus* 0/18 from inactivation-shift (P<0.05). In 11 rabbit atrial myocytes in which EADs were generated either by increasing conductance of normal-window-width $I_{CaL,D\text{-}C}$ or subsequent 4-aminopyridine (2 mM), window-$I_{CaL,D\text{-}C}$-narrowing (10 mV) abolished EADs of all types (P<0.05).

Conclusion: We validated the dynamic-clamp for I_{CaL}, novel in atrial cardiomyocytes, and showed that EADs of various types are generated by widening (particularly asymmetrically) window-I_{CaL}, and abolished by narrowing it. Window-I_{CaL}-narrowing is a potential therapeutic mechanism worth pursuing in the search for improved anti-AF drugs.

P43: **A novel regulatory pathway for transient receptor potential melastatin 7 (TrpM7), a new player in hypertension**

Authors: **GAO XING**,. William Fuller

University of Glasgow

Altered magnesium homeostasis has been reported to contribute to the pathophysiology of hypertension, a major risk factor for morbidity and mortality in developed and developing countries and the leading cause of cardiovascular disease. Transient receptor potential melastatin 7 (TrpM7) is a bifunctional protein comprising a cation channel and a functional serine/threonine kinase at its carboxyl terminus domain. The ion channel controls transmembrane magnesium (Mg) flux, while the kinase domain controls downstream intracellular signalling pathways, as well as regulating the channel itself. Abnormal functioning of the channel and kinase domains in TrpM7 has been associated with Mg dysregulation, vascular dysfunction and elevated blood pressure, highlighting the importance of studying the cellular processes involved in its activity and regulation. TrpM7 is regulated by aldosterone and has recently been implicated in the development of angiotensin-II induced hypertension.

Palmitoylation, the reversible conjugation of a 16-carbon fatty acid to cysteine side chains, regulates the activity and subcellular localisation of numerous ion transporters in the cardiovascular system. TrpM7 is palmitoylated. This project aims to map its palmitoylation sites, research the impact of TrpM7 regulatory pathways on TrpM7 palmitoylation, and identify the functional impact of palmitoylation on TrpM7. In addition, we will evaluate TrpM7 palmitoylation in established models of vascular dysfunction, aiming to shed light on a new player in the pathogenesis of cardiovascular disease.

P44: **Morphological and histological features of the Greenland shark coronary circulation**

Authors: Sana Yaar, Jennifer Thomson, Alison Gurney, Holly Shiels

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The Greenland shark (Somniosus microcephalus) is a cold-water elasmobranch that exhibits extremely low growth rates, blood pressures, and swim speed for its size [>5m]. It has the longest known lifespan of any vertebrate, reaching sexual maturity at 150 years and surviving beyond 270 years. The physiological features that support such longevity remain to be determined. Here we investigated whether the coronary vasculature of the Greenland shark heart showed signs of dysfunction normally associated with aging in humans. We studied the coronary arteries in post mortem heart samples from 8 Greenland sharks (age range 99-212 years), captured off the south east coast of Greenland. Tissue sampling adhered to authorisation from the Greenland government. Vascular structures in paraffin-embedded, fixed and sectioned tissues of the different ventricular layers (epicardium, compact, and spongiosa) were identified using common histological and immunohistochemical staining techniques and bright field and polarised light microscopy. Parameters measured included: the density of coronary micro-vessels, inter-vessel distances, the ratio of arteriole wall thickness to lumen diameter, the content of collagen and elastic fibres and cell proliferation. The arteries were also examined for evidence of lesions, such as atheroma, intimal or medial hyperplasia. We did not detect an age-related trend in any parameter in any ventricular layer. Moreover, we found no obvious signs of coronary artery disease, even in the oldest shark. Maintained coronary health may contribute to a healthy heart and a long life in the Greenland shark.

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28th Northern Cardiovascular Research Group Meeting
Bradford 2020

Thank you from everyone at The University of Leeds, Faculty of Biological Sciences. We hope you found the day both enjoyable and beneficial. We will see you all next year for the 28th Northern Cardiovascular Research Group meeting, which will be hosted by the University of Bradford in collaboration with Dr. Matt Hardy, Dr. Kirsten Riches-Suman and Dr. Jacob Elies Gomez

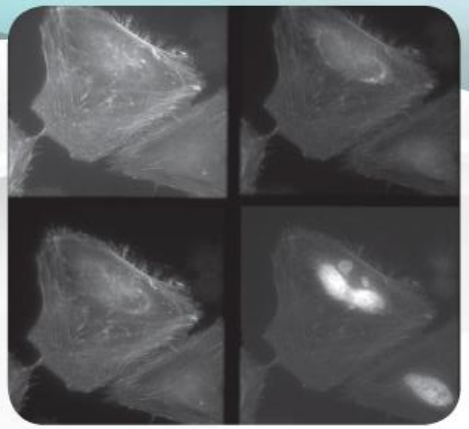
Thank you from

Dr. Hannah M. Kirton
&
The Faculty of Biological Sciences Cardiovascular team

NOTES:

MULTISPLIT V2 4 images on a single camera chip

Introducing the latest member of our image splitting family



- Ratiometric Calcium
- Voltage
- FRET
- Multi-depth imaging

The home of the image splitter

CAIRN RESEARCH

Images courtesy of Dr. Joachim Goedhart & Marieke Mastop, University of Amsterdam

For more information on our MultiSplit, visit https://www.cairn-research.co.uk/product/multisplit-v2/