3 AGEING OF THE VASCULAR ENDOTHELIUM: A NOVEL ROLE FOR CAMKIIδ?

Claire McCluskey*, Margaret MacDonald, Susan Currie. *Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, UK*

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Calcium/calmodulin–dependent protein kinase II delta (CaMKIIδ) plays a pivotal role in cardiovascular health and disease. Whether CaMKIIδ modulates impaired function in ageing cardiovasculature is unknown. This study investigates cardiac and aortic CaMKIIδ expression and activation in aged rats. Cardiovascular function was assessed *in vivo* by echocardiography. Reduced fractional shortening (63.2±1.3 vs 50.6 ±1.9 (%FS), young vs aged (n=12), $p<0.01$) and increased heart weight:body weight (2.7±0.2 vs 3.6±0.1, young vs aged (n=6), $p<0.05$) were observed in aged animals suggesting cardiac remodelling. Increased blood flow through the ascending aorta was also observed in aged rats (76.7±3.1 vs 103.4±7.4 (ml/min), young vs. aged (n=12), $p<0.05$). Parallel investigation of CaMKIIδ in cardiac and aortic tissue revealed increased protein expression (1.03±0.1 vs11.7±0.2, (n=7) $p<0.05$ (cardiac); 1.1±0.1 vs. 1.3±0.1 (aortic), young vs. aged (n=6) (CaMKIIδ/GAPDH)). Further analysis of aortic tissue showed increased activation of CaMKII with elevations in phosphorylated and oxidised CaMKII (ox-CaMKII) expression (1.2±0.1 vs 1.8±0.2 (n=5) $p<0.05$; 1.0±0.1 vs 1.3±0.08 (n=5) $p<0.05$, young vs. aged) as well as increased kinase activity (5.9±1.2 vs 8.2±1.4 ($pmolPO_4^-inc/min/\mu g$ protein) young vs.aged, (n=3)). To explore these novel observations in the vasculature further, endothelial cells from young and aged aortae were isolated. These cells displayed striking phenotypic differences between groups with aged cells displaying clear signs of necrosis. To investigate whether ox-CaMKII may contribute to deterioration of the endothelium in ageing, initial experiments studied young endothelial cells exposed to H_2O_2 (10 μM). Reactive oxygen species and ox-CaMKII expression were measured in parallel and a corresponding increase in both parameters occurred following treatment (2.5×10^3 vs 4.4×10^4 (fluorescence a.u.) control vs treated, (n=3), $p<0.01$; 1.1±0.2 vs 1.82±0.1 (ox-CaMKII/GAPDH), control vs treated), $p<0.001$, (n=3)). This work provides new evidence that CaMKIIδ expression and activation are augmented in aged vasculature. Future work will examine CaMKII in aged endothelial cells to provide mechanistic insight into the deterioration of vascular function.

4 THE ROLE OF INTRACELLULAR CA^{2+} ON THE ARRHYTHMOGENIC ACTIVITY OF PULMONARY VEIN CARDIOMYOCYTES

[1]Alasdair D Henry*, [2]Niall MacQuaide, [2]Andrew C Rankin, [1]Edward G Rowan, [1]Robert M Drummond. *[1]University of Strathclyde, Strathclyde Institute of Pharmacy and Biomedical Sciences, Glasgow, UK; [2]University of Glasgow, Institute of Cardiovascular and Medical Sciences, College of Medical, Veterinary and Life Sciences, Glasgow, UK*

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The Pulmonary veins (PVs) are a widely recognised source of ectopic electrical activity that can lead to atrial fibrillation. While this activity likely originates from the external sleeve of cardiomyocytes that surround the PV, the underlying mechanisms are currently unknown. Changes in intracellular Ca^{2+} signalling are thought to influence the electrical properties of PV cardiomyocytes; therefore the PV was isolated from the rat and intracellular Ca^{2+} was monitored with the fluorescent indicator fluo-4. Spontaneous Ca^{2+} transients were present under resting conditions and typically manifested as waves occurring asynchronously between neighbouring cells. Immediately following a brief period of electrical field stimulation (EFS) at 3 Hz or greater, the frequency of the spontaneous Ca^{2+} transients was increased. Furthermore, this effect was more pronounced after the extracellular Ca^{2+} concentration was increased. Further analysis showed that the spontaneous Ca^{2+} transients occurred more synchronously in the initial few seconds following electrical stimulation at 3 to 9 Hz. As spontaneous Ca^{2+} transients are due to Ca^{2+} released from the sarcoplasmic reticulum through the ryanodine receptors, immunocytochemistry was used to determine their distribution. The ryanodine receptors were arranged in a striated pattern in the cell interior and also along the periphery of cell, which was similar to atria myocytes. However, unlike atrial cells, labelling of the membrane with Di-4 ANEPPS revealed the presence of t-tubules in PV cardiomyocytes. When the contractile response of the PV was studied *in vitro*, contractions that were ~50–80 ms in duration, which is typical of cardiac tissue, could be evoked by electrical stimulation. The α and β adrenoceptor agonist noradrenaline induced periodic bursts of contractions that occurred independently of electrical stimulation and this arrhythmogenic activity was supressed by inhibiting the Na^+/Ca^{2+} exchanger (NCX) with ORM-10103, suggesting an important role for the NCX in noradrenaline induced automaticity.

5 ASSESSING THE ROLE OF EXTRACELLULAR VESICLES IN RENIN-ANGIOTENSIN SYSTEM SIGNALLING INCARDIOMYOCYTE HYPERTROPHY

[1]Laura S Downie*, [2]Katrin Nather, [1]Lorraine M Work, [1]Stuart A Nicklin. *[1]Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK; [2]Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK*

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Introduction The classical Renin-Angiotensin System has been implicated in cardiovascular remodelling including cardiac hypertrophy while a counter-regulatory axis of the RAS has been shown to have cardioprotective effects against hypertrophy. Here, we show the characterisation of cardiac-derived extracellular vesicles (EVs) from two different cardiac cell types, neonatal rat cardiac fibroblasts (NRCF) and H9c2 cardiomyocytes. We suggest that these EVs may be harnessed for delivery of peptides involved in RAS signalling further leading to either cardioprotective or deleterious effects.

Methods EVs were isolated from NRCF cell or H9c2 cardiomyocyte conditioned media via differential centrifugation/ultracentrifugation and concentration and size was verified by BCA, nanosight and transmission electron microscopy (TEM). H9c2 cardiomyocytes were left untreated (control) or treated with 1 μM AngII or 1 μM Ang-(1-7) soluble peptide for 48 hours. EVs were isolated and placed onto recipient H9c2 cardiomyocytes for 96 hours, phalloidin stained cells were then imaged by confocal microscopy and sized on Image J for analysis of hypertrophy.

Results EVs isolated from NRCF cells are larger and released at higher concentrations than those isolated from H9c2 cardiomyocytes (NRCF EVs 200 nm size, 3.5×10^8 particles/ml

vs. H9c2 EVs 100 nm size, 2.5 × 10^8 particles/ml). EVs isolated from 1 μM AngII treated H9c2 cardiomyocytes significantly increased cell area of recipient H9c2 cardiomyocytes (control EV treated 3291.1±90.1 μm^2 vs AngII EV treated 5252.3±125.4 μm^2, p<0.001). EVs isolated from 1 μM Ang-(1-7) treated H9c2 cardiomyocytes significantly reduced AngII peptide induced increase in cell area in a dose dependent manner in recipient H9c2 cardiomyocytes (100 nM AngII +Control EVs 5566.3±139.0 μm^2 vs 100 nM AngII+Ang-(1-7) EVs 4212.7±132.1 μm^2, p<0.001).

Summary/conclusions Collectively these data suggest that there are distinct sub-populations of EVs released that differ between NRCF cells and H9c2 cardiomyocytes. We have shown that H9c2-derived EVs can elicit deleterious or cardioprotective effects on recipient H9c2 cardiomyocytes depending on the condition of the parental cells.

6 RESISTIN MEDIATES SEX-DEPENDENT EFFECTS OF PERIVASCULAR ADIPOSE TISSUE ON VASCULAR FUNCTION IN THE SHRSP

[1]Sarah McNeilly*, [1]Heather Y Small, [1,2]Sheon Mary, [1]Adam Sheikh, [1]Christian Delles. *[1]BHF Glasgow Cardiovascular Research Centre, Institute of Cardiovascular and Medical Sciences, University of Glasgow, Scotland, UK; [2]Department of Biochemical Sciences, CSIR-National Chemical Laboratory, India*

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Introduction Premenopausal women are relatively protected against hypertension compared to males. Oestrogen levels have been identified as a potential underlying cause, but many of the pathophysiological mechanisms remain to be determined. Altered perivascular adipose tissue (PVAT) function has been identified to have vasoactive effects. However, in hypertension, sex-dependent differences of PVAT have not yet been explored.

Hypothesis Sex-dependent effects of PVAT mediate altered vascular function in hypertension.

Approach and result The effect of PVAT was investigated on resistance vessels of 16 week old male and female stroke-prone spontaneously hypertensive rats (SHRSP). This preclinical model of hypertension presents with a sex-difference in the development of hypertension comparable to humans. Wire-myography was used on 3rd order mesenteric vessels to assess vascular function. Noradrenaline mediated vasoconstriction was increased in SHRSP males compared to females (maximum contraction: male +PVAT 113.3±1.1% vs female +PVAT 91.4%±11.36%). K_{ATP} channel-mediated vasorelaxation by cromakalim was impaired in males compared to females (maximum relaxation: male +PVAT 46.9±3.9% vs female +PVAT 97.3%±2.7%). A cross-over study assessing function of male PVAT on female vessels and vice versa confirmed the reduced K_{ATP} mediated vasorelaxation induced by male PVAT (maximum relaxation: female +$PVAT_{female}$ 90.6 ±1.4% vs female +$PVAT_{male}$ 65.8%±3.5%). To explore the cause of sex-dependent differences in PVAT an adipokine array with subsequent western blot validation was carried out. This identified resistin as a potential modifier of vascular reactivity. Resistin was increased by approximately 2-fold in SHRSP male mesenteric PVAT. Further wire-myography experiments with male and female vessels pre-treated with resistin (40 ng/ml) showed no difference in response to noradrenaline. However, vasorelaxation in response to cromakalim was significantly impaired in resistin treated female vessels, similar to levels observed in male vessels (maximum relaxation: female +PVAT 97.3±0.9% vs female +PVAT +resistin[40 ng/ml] 36.8% ±2.3%).

Conclusion These findings indicate a novel role for resistin in sex-dependent PVAT mediated vascular function in hypertension through a K_{ATP} channel mediated mechanism.

7 QUANTIFYING THE BIO-DISTRIBUTION OF TRANSPLANTED HESC-ECS IN A MURINE MODEL OF HLI BY QPCR

[1]Lucija Fleisinger*, [1]Mark G MacAskill, [2]Joanne C Mountford, [1]Andrew H Baker, [1]David E Newby, [1]Patrick WF Hadoke. *[1]University/BHF Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, UK; [2]Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK*

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Introduction Beneficial effects of stem/progenitor cell therapies for the treatment of ischaemic diseases have been established; however, there remains conflicting evidence on whether cells act by incorporation into the existing vasculature or by paracrine effects.

Objective This study aims to quantify the bio-distribution of transplanted, proangiogenic, human embryonic stem cell-derived endothelial cells (hESC-ECs) in a murine model of hind limb ischaemia over 21 days using qPCR-detection of human DNA.

Methods CD1 nude mice (male, 6–8 weeks old; n=6 per group) underwent femoral artery ligation followed by injection with hESC-ECs into ischaemic muscle of the hindlimb. Mice were sacrificed at 0 hour, 4 hour, 24 hour, 7d, 14d, and 21d post-transplantation, and DNA extracted from the hind limb. A paired-qPCR assay was run with a set of human-specific and mouse-specific primers to allow for detection of hESC-ECs in mouse tissue.

Results A standard curve was constructed using mixtures of DNA extracted from mouse tissue and a population of hESC-ECs. The mean percentage of human cells at each time-point was determined accordingly. At the first time-point (0 hour), 1.77%±0.55% human cells were present within the ischaemic limb, with no significant change at 4 hour post-injection. However, by 24 hour and 7 days post-injection, the% human cells present significantly decreased to 0.67%±0.24% (p=0.05) and 0.35%±0.09% (p=0.05) respectively. At 14 and 21 days post-injection, the level of human DNA present had decreased to background levels.

Conclusion Our results suggest the majority of injected hESC-EC cleared from the injection site within the first 24 hours, with the remainder of the cells no longer present at 14 days post-injection. This is consistent with imaging data obtained prior to this study, and suggests that injected hESC-ECs improve perfusion in the mouse ischaemic limb by a paracrine mechanism rather than direct incorporation into the existing vasculature.