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

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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

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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

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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