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Ventricular pro-arrhythmic phenotype, arrhythmic substrate, ageing and mitochondrial dysfunction in peroxisome proliferator activated receptor-γ coactivator-1β deficient (*Pgc-1β*$^{-/-}$) murine hearts

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ABSTRACT

Introduction: Ageing and age-related bioenergetic conditions including obesity, diabetes mellitus and heart failure constitute clinical ventricular arrhythmic risk factors.

Materials and methods: Pro-arrhythmic properties in electrocardiographic and intracellular recordings were compared in young and aged, peroxisome proliferator-activated receptor-γ coactivator-1β knockout (*Pgc-1β*$^{-/-}$) and wild type (WT), Langendorff-perfused murine hearts, during regular and programmed stimulation (PES), comparing results by two-way ANOVA.

Results and discussion: Young and aged *Pgc-1β*$^{-/-}$ showed higher frequencies and durations of arrhythmic episodes through wider PES coupling-interval ranges than WT. Both young and old, regularly-paced, *Pgc-1β*$^{-/-}$ hearts showed slowed maximum action potential (AP) upstrokes, $(dV/dt)_{max}$ (~157 *vs.* 120–130 V s^{-1}), prolonged AP latencies (by ~20%) and shortened refractory periods (~58 *vs.* 51 ms) but similar AP durations (~50 ms at 90% recovery) compared to WT. However, *Pgc-1β*$^{-/-}$ genotype and age each influenced extrasystolic AP latencies during PES. Young and aged WT ventricles displayed distinct, but *Pgc-1β*$^{-/-}$ ventricles displayed similar dependences of AP latency upon $(dV/dt)_{max}$ resembling aged WT. They also independently increased myocardial fibrosis. AP wavelengths combining activation and recovery terms paralleled contrasting arrhythmic incidences in *Pgc-1β*$^{-/-}$ and WT hearts. Mitochondrial dysfunction thus causes pro-arrhythmic *Pgc-1β*$^{-/-}$ phenotypes by altering AP conduction through reducing $(dV/dt)_{max}$ and causing age-dependent fibrotic change.

1. Introduction

Cardiovascular disease is the leading worldwide cause of mortality. Approximately half such cases are attributable to sudden cardiac death (SCD) (Go et al., 2013), often following ventricular arrhythmias. The latter follow disruption of the normally coordinated sequence of activation and inactivation of ion channel species underlying cardiac action potentials (AP). Models for several *monogenic* ion channel disorders using genetically-modified murine hearts have provided valuable insights into the contributions of particular channels to arrhythmic events (Huang, 2017). However, such conditions account for a relatively small proportion of SCDs in the clinical setting. Growing evidence also links such arrhythmias to energetic dysfunction seen in both ageing and age-related conditions including obesity, diabetes mellitus and heart failure (Hookana et al., 2011; Kucharska-Newton et al., 2010; Yeung et al., 2012). The latter constitute risk factors for SCD independent of any underlying coronary artery disease (Adabag et al., 2015; Yeung et al., 2012). Ageing itself is associated with an increased incidence of cardiac rhythm disturbances including both pathological bradycardic rhythms as well as atrial and ventricular tachy-arrhythmias (Bradshaw et al., 2014; Deo and Albert, 2012; Go et al., 2001), though the underlying mechanisms remain unclear.

Biochemical consequences of energetic deficiency have been studied in systems deficient in peroxisome proliferator activated receptor-γ coactivator-1 (PGC-1) transcriptional coactivators. These proteins regulate mitochondrial mass, function and cellular metabolism, upregulating expression of nuclear and mitochondrial genes involved in fatty acid β–oxidation, the tricarboxylic acid cycle and electron transport

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(Arany et al., 2005). In particular, PGC-1α and PGC-1β are highly expressed in oxidative tissues such as the heart, serving to co-ordinate mitochondrial activity with upstream cellular signals (Sonoda et al., 2007). They thus form a nexus for a range of metabolic pathways within the cardiomyocyte, central to the heart's ability to meet energetic demands. Their expression is down-regulated in obesity, insulin resistance and type II diabetes mellitus along with an associated mitochondrial dysfunction (Dillon et al., 2012). Mice deficient in *both Pgc-1α* and *Pgc-1β* develop a perinatally lethal, low cardiac output state and conduction disease (Lai et al., 2008). In contrast, both *Pgc-1α*$^{-/-}$ and *Pgc-1β*$^{-/-}$ hearts show normal baseline function (Arany et al., 2005; Lelliott et al., 2006), with *Pgc-1β*$^{-/-}$ hearts displaying abnormal electrophysiological responses to adrenergic challenge. Together with its normal baseline contractile function these features make *Pgc-1β*$^{-/-}$ models attractive to investigating pro-arrhythmic effects of *chronic* mitochondrial dysfunction.

Cellular electrophysiological abnormalities have also been associated with energetic dysfunction. Firstly, an increased production of reactive oxygen species (ROS) affects maximum voltage-dependent Na^+ and K^+ current, Na^+ and Ca^{2+} channel inactivation, late Na^+ current (Liu et al., 2010; Wang et al., 2004), and ryanodine receptor and gap junction function (Brown and O'Rourke, 2010; Sovari et al., 2013; Terentyev et al., 2008). Secondly, ATP/ADP depletion accompanying mitochondrial dysfunction increases sarcolemmal ATP-sensitive K^+ channel ($sarcK_{ATP}$) open probabilities, shortening AP duration (Fischbach et al., 2004). Thirdly, oxidative stress and increased ROS formation may promote fibrotic change (Dai et al., 2009; Hafner et al., 2010), possibly through increased TGF-β activity (Brooks and Conrad, 2000; Rosenkranz et al., 2002), potentially disrupting gap junction function (Chilton et al., 2007; van Veen et al., 2005; Xie et al., 2009). Accordingly, studies in isolated *Pgc-1β*$^{-/-}$ cardiomyocytes specifically reported altered ion channel expression and function, abnormal Ca^{2+} homeostasis and delayed afterdepolarisation phenomena (Gurung et al., 2011).

These cellular changes have provided potential mechanisms altering cell-cell coupling (Smyth et al., 2010), AP conduction (Liu et al., 2010), repolarisation and refractoriness (Wang et al., 2004), and Ca^{2+}-mediated triggering phenomena (Terentyev et al., 2008) in monogenic ion channel arrhythmic models. Structural abnormalities appearing as age-related fibrosis (Jeevaratnam et al., 2012, 2011), or compromised Na^+ current activation through Nav1.5 deficiency proved pro-arrhythmic in *Scn5a*$^{+/-}$ (Jeevaratnam et al., 2012, 2011; Martin et al., 2012) and *Scn5a*$^{+/\Delta KPQ}$ hearts (Wu et al., 2012) through altered AP activation and conduction. Similarly AP *recovery* abnormalities proved pro-aarrhythmic in *Scn5a*$^{+/\Delta kpq}$ and *Kcne5*$^{-/-}$ models for long QT syndromes (Sabir et al., 2008; Thomas et al., 2008, 2007). Altered intracellular Ca^{2+} homeostasis in *RyR2-P2328S* hearts both compromised Na^+ currents (King et al., 2013b; Ning et al., 2016) and produced early or delayed afterdepolarization triggering events (Goddard et al., 2008; Hothi et al., 2008).

However, relatively few studies have investigated the electrophysiological consequences of these cellular changes and their implications for arrhythmic triggering or arrhythmic substrate at the level of *intact Pgc-1β*$^{-/-}$ hearts. Such hearts have shown potentially pro-arrhythmic APD alternans phenomena, and increased frequencies of ventricular tachycardia (VT) (Gurung et al., 2011; Lelliott et al., 2006), particularly with explorations through different steady-state pacing rates, the latter particularly in aged *Pgc-1β*$^{-/-}$ hearts. These were associated with reduced maximum action potential (AP) upstroke velocities, $(dV/dt)_{max}$ and increased AP conduction latencies (Ahmad et al., 2017).

The present experiments characterise the electrophysiological mechanisms underlying arrhythmic substrates underlying these changes and how these progress with age in *Pgc-1β*$^{-/-}$ hearts modeling chronic mitochondrial dysfunction. They compared four groups of intact, young and aged, wild type (WT) and genetically modified, Langendorff-perfused *Pgc-1β*$^{-/-}$ hearts. Triggering events provoking arrhythmia in the presence of substrate were mimicked by S2 stimuli interposed at differing intervals following regular S1 pacing trains following protocols established on earlier occasions (Thomas et al., 2008, 2007). Direct intracellular determinations of resting membrane potentials (RMPs), AP amplitudes and latencies, and maximum rates of AP depolarisation, $(dV/dt)_{max,}$ in cardiomyocytes *in situ* ensured unperturbed intracellular conditions, particularly of Ca^{2+} homeostasis. *Pgc-1β*$^{-/-}$ as opposed to WT genotypes were implicated in decreased $(dV/dt)_{max}$ and increased AP latencies in the absence and in the presence of effects of age respectively. The latter segregation prompted explorations demonstrating distinct dependences of AP latency on $(dV/dt)_{max}$ in young and aged WT hearts but a single such dependence in both *Pgc-1β*$^{-/-}$ groups approximating the functions observed in aged WT. The difference could be accounted for effects on AP latency of increases in fibrotic change arising from both *Pgc-1β*$^{-/-}$genotype and ageing. Predictions of arrhythmic substrate from wavelengths derived from these AP activation and recovery terms, paralleled the relative incidences of arrhythmia in *Pgc-1β*$^{-/-}$ and WT hearts.

2. Materials & methods

2.1. Experimental animals

This research has been regulated under the Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012 following ethical review by the University of Cambridge Animal Welfare and Ethical Review Body (AWERB). Age-matched homozygote *Pgc-1β*$^{-/-}$ and WT inbred C57/B6 mice were studied, with alternate male and female animals used in successive experiments within each group. *Pgc-1β*$^{-/-}$ mice were generated using a triple LoxP targeting vector as previously described (Lelliott et al., 2006). The young WT and young *Pgc-1β*$^{-/-}$ groups consisted of mice aged between 3–4 months; animals aged greater than 12 months were used for the aged WT and aged *Pgc-1β*$^{-/-}$ groups respectively. Mice were housed in plastic cages maintained at 21 ± 1 °C, subjected to 12 h dark/light cycles, and had unconstrained access to water, sterile rodent chow (RM3 Maintenance Diet, SDS, Witham, Essex, UK), bedding and environmental stimuli.

2.2. Whole heart Langendorff preparations

All chemical agents were purchased from Sigma-Aldrich (Poole, UK) except where otherwise indicated. Mice were first anticoagulated with 200 IU heparin sodium administered into the intra-peritoneal space with a 27 G hypodermic needle. After a 10 min interval, mice were killed by cervical dislocation (Schedule 1: UK Animals (Scientific Procedures) Act (1986)), a sternotomy performed, and the hearts were rapidly excised and submerged in ice-cold KH solution. The aorta was then cannulated with a modified 21 G hypodermic needle, secured with a 5-0 braided silk suture and retrogradely perfused with Krebs-Henseleit (KH) solution warmed to 37 °C by a water jacket heat-exchange coil (model C-85 A, Techne, Cambridge, UK) at a constant rate of 2.05 ml min^{-1} by a peristaltic pump (MINIPULS3, Gilson, Luton, UK) through 200 μm and 5 μm Millipore filters (Millipore, Watford, UK). The KH buffer was made with NaCl (119 mM), $NaHCO_3$ (25 mM), KCl (4 mM), $MgCl_2$ (1 mM), KH_2PO_4 (1.2 mM), $CaCl_2$ (1.8 mM), glucose (10 mM) and sodium pyruvate (1.8 mM), bubbled with 95% O_2/5% CO_2 (British Oxygen Company, Manchester, UK) to achieve a pH of 7.4 preventing $CaCO_3$ precipitation and matching the 7.3–7.4 pH of mouse plasma. Following commencement of perfusion, preparations were only further studied if they demonstrated sustained intrinsic activity with a basic cycle length (BCL) < 200 ms and 1:1 atrioventricular conduction (AV) for 10 min. Hearts meeting these criteria were then perfused with 150 ml KH solution containing 20 μM blebbistatin after which perfusion with plain KH solution continued through to the conclusion of the experiment. The blebbistatin (20 μM, Selleckchem, Houston, USA) was