Journal of Molecular and Cellular Cardiology 94 (2016) 54–64

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Prolongation of atrio-ventricular node conduction in a rabbit model of ischaemic cardiomyopathy: Role of fibrosis and connexin remodelling

Ashley M. Nisbet [a,1], Patrizia Camelliti [b,*,1], Nicola L. Walker [a], Francis L. Burton [a], Stuart M. Cobbe [a], Peter Kohl [c,d], Godfrey L. Smith [a,*]

[a] British Heart Foundation Glasgow Cardiovascular Research Centre, University of Glasgow, Glasgow G12 8QQ, UK
[b] School of Biosciences and Medicine, University of Surrey, Guildford, GU2 7XH, UK
[c] Institute for Experimental Cardiovascular Medicine, University Heart Centre Freiburg - Bad Krozingen, Medical School of the University of Freiburg, Germany
[d] Heart Science Centre, National Heart and Lung Institute, Imperial College London, Harefield UB9 6JH, UK

A R T I C L E I N F O

Article history:
Received 17 November 2015
Received in revised form 16 March 2016
Accepted 23 March 2016
Available online 25 March 2016

Keywords:
Myocardial infarction
Heart failure
Left ventricular dysfunction
Conduction
Optical mapping
Connexins

A B S T R A C T

Conduction abnormalities are frequently associated with cardiac disease, though the mechanisms underlying the commonly associated increases in PQ interval are not known. This study uses a chronic left ventricular (LV) apex myocardial infarction (MI) model in the rabbit to create significant left ventricular dysfunction (LVD) 8 weeks post-MI. *In vivo* studies established that the PQ interval increases by approximately 7 ms (10%) with no significant change in average heart rate. Optical mapping of isolated Langendorff perfused rabbit hearts recapitulated this result: time to earliest activation of the LV was increased by 14 ms (16%) in the LVD group. Intra-atrial and LV transmural conduction times were not altered in the LVD group. Isolated AVN preparations from the LVD group demonstrated a significantly longer conduction time (by approximately 20 ms) between atrial and His electrograms than sham controls across a range of pacing cycle lengths. This difference was accompanied by increased effective refractory period and Wenckebach cycle length, suggesting significantly altered AVN electrophysiology post-MI. The AVN origin of abnormality was further highlighted by optical mapping of the isolated AVN. Immunohistochemistry of AVN preparations revealed increased fibrosis and gap junction protein (connexin43 and 40) remodelling in the AVN of LVD animals compared to sham. A significant increase in myocyte–non-myocyte connexin co-localization was also observed after LVD. These changes may increase the electrotonic load experienced by AVN muscle cells and contribute to slowed conduction velocity within the AVN.
© 2016 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

The prognosis in chronic heart failure (CHF) is affected by conduction abnormalities in both the atrio-ventricular node (AVN) and the His-Purkinje system in approximately 50% of patients [1–5]. Atrio-ventricular and intra-ventricular conduction changes can produce adverse haemodynamic effects *via* their impact on left ventricular (LV)/right ventricular (RV) synchrony and ventricular contraction-relaxation sequence. Slower atrio-ventricular conduction manifests itself on the surface electrocardiogram (ECG) *via* a prolonged PR interval. This leads to delayed ventricular activation which may be sufficient to cause pre-systolic mitral regurgitation, reducing LV preload and, hence, output. Multisite biventricular pacing techniques (also known as cardiac resynchronisation therapy) improve cardiac hemodynamic function by correcting LV and RV activation times [6–8]. Further improvements in systolic function can be achieved by optimisation of preload by correct timing of atrio-ventricular delay [2,9,10]. The causes and mechanisms of abnormal conduction are not known. In particular, whether a specific site in the conduction system is involved, and whether the effect is a direct or indirect consequence of a pathological change, are open questions. That said, a recent publication reported both structural and molecular changes within the AVN of a rabbit model of cardiac hypertrophy [11], suggesting that this tissue region may be causally involved.

Physiological conduction in the AVN is already slow, compared to atrial and ventricular myocardium, due to distinct electrical properties of AVN tissue, including significantly different expression levels of a range of ion channels, including connexins [12]. The mammalian heart contains three main connexin isoforms: connexin43 (Cx43), connexin40 (Cx40) and connexin45 (Cx45). There is heterogeneous expression of all three isoforms within the tissue of the Triangle of Koch [13]. The most abundant cardiac connexin, Cx43, has major roles in

Abbreviations: AVN, atrio-ventricular node; AN, atrio-nodal; CHF, chronic heart failure; ERP_A, atrial effective refractory period; ERP_{AVN}, AVN effective refractory period; FRP_{AVN}, AVN functional refractory period; LV, left ventricle; LVD, left ventricular dysfunction; MI, myocardial infarction; NH, nodo-Hisian; O/N, overnight; RV, right ventricle; RT, room temperature; Tact, activation time.

* Corresponding authors.
E-mail address: p.camelliti@surrey.ac.uk (P. Camelliti).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.yjmcc.2016.03.011
0022-2828/© 2016 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

cell-cell communication of working ventricular and atrial myocytes. It shows relatively low expression within the compact AVN, but is observed in the transitional zones of the atrio-nodal (AN) and nodo-Hisian (NH) regions. The posterior nodal extension has the lowest Cx43 mRNA and the most abundant HCN4 mRNA levels, in keeping with its low conduction velocity and secondary pacemaker activity [14]. In contrast, the low-conductivity Cx45 has been shown to be abundant in the compact node, and both Cx40 and Cx45 have been reported in the NH region [15–17].

The mechanisms underlying abnormal delays in atrio-ventricular conduction in CHF are not fully understood. This study therefore aims to assess atrio-ventricular conduction delay in a rabbit model of left ventricular dysfunction (LVD) due to apical myocardial infarction (MI), and to investigate possible mechanisms underlying this delay. Our results indicate that the significantly longer PQ interval, observed in this rabbit model of LVD, is due to abnormally slow conduction through the compact AVN. The increase in conduction time is associated with fibrosis, higher non-myocyte content and altered expression of connexins in the AVN, possibly including hetero-typic cell coupling, as part of the structural remodelling following MI.

2. Methods

2.1. Animal model

Procedures were undertaken in accordance with the United Kingdom Animals (Scientific Procedures) Act of 1986 and conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996). A well-characterised model of MI, induced by coronary artery ligation, was used [18–24]. In short, adult male New Zealand White rabbits (2.5–3.0 kg) were given premedication with 0.4 mL/kg intramuscular Hypnorm (fentanyl citrate, 0.315 mg/mL: fluanisone 10 mg/mL, Janssen Pharmaceuticals). Anaesthesia was induced with 0.25–0.5 mg/kg midazolam (Hypnovel, Roche) given *via* an indwelling cannula in the marginal ear vein. Rabbits were intubated and ventilated using a Harvard small animal ventilator with a 1:1 mixture of nitrous oxide and oxygen containing 1% halothane at a tidal volume of 50 mL and a frequency of 40 min^{-1}. Preoperative antibiotic prophylaxis was given with 1 mL Amfipen (ampicillin 100 mg/mL, Mycofarm UK Ltd) intramuscularly. A left thoracotomy was performed through the 4th intercostal space. Quinidine hydrochloride (10 mg/kg; Sigma Pharmaceuticals), a class IA antiarrhythmic (potassium channel blocker) was administered intravenously prior to coronary artery ligation to reduce the incidence of ventricular fibrillation. The marginal branch of the left circumflex coronary artery, which supplies most of the LV free wall, was ligated halfway between the atrio-ventricular groove and the cardiac apex to produce an ischaemic area of 30–40% of the LV. As there is relatively little collateral circulation in the rabbit, a relatively uniform apical MI was produced transmurally, occupying on average 14% of the total endocardial and epicardial surfaces of the LV (Fig 1A(i)) [18]. Sham controls did not undergo coronary artery ligation, but were subjected to all other interventions.

2.2. Characterisation of the rabbit model of LVD

Rabbits were sedated with 0.3 mg/kg Hypnorm prior to echocardiography and ECG. Echocardiographic assessment of LV end-diastolic dimension and systolic function was performed at 7 weeks post coronary artery ligation or sham operation, using a 5 MHz paediatric probe with a Toshiba sonograph (Sonolayer 100). The ECG was measured to determine RR and PQ intervals, and QS and QT durations in the intact animal, and to identify any effect of LVD on these parameters. PQ and QS intervals were reported instead of PR in an attempt to assess the contribution of ventricular conduction time separately from atrial and AVN conduction. To record high quality ECG signals, three electrodes were positioned subcutaneously, and the ECG recorded from lead II. Increased inducibility of arrhythmias and lowered ventricular fibrillation threshold were further assessed after organ isolation, *ex vivo*, 8 weeks after the initial surgery [18].

This study describes investigations both *in vivo* and *in vitro*. Three distinct *in vitro* studies were performed: (i) optical measurements on isolated whole hearts (8 sham and 8 LVD preparations); (ii) extracellular electrode measurements on isolated AVN preparations (14 sham and 14 LVD); (iii) optical measurements on isolated AVN preparations (4 sham and 4 LVD). In a separate set of experiments, ECG measurements were made on sham and LVD rabbit groups as described above (8 sham and 8 LVD animals). Accumulated mortality statistics, excluding mortality associated with the operative procedure, were collected over approximately 20 years of studies using the rabbit MI model (499 LVD and 303 sham procedures).

2.3. Whole heart optical mapping studies

Eight weeks after surgery, rabbits (8 LVD, 8 sham controls) were sacrificed with an intravenous injection of 0.5 mL/kg Euthatal (sodium pentobarbitone 200 mg/kg, Rhone Merieux), mixed with 500 IU of heparin. Hearts were rapidly excised and Langendorff-perfused with oxygenated Tyrode's solution (containing in mmol/L: NaCl 93, $NaHCO_3$ 20, Na_2HPO_4 1.0, $MgSO_4$ 1.0, KCl 5.0, $CaCl_2$ 1.8, Na-acetate 20, and glucose 20; equilibrated with 95% O_2/5% CO_2, pH 7.4.) at 37 °C and at constant rate of 40 mL/min using a Gilson Minipuls 3 peristaltic pump. Perfusion pressure was monitored with a transducer in the aortic cannula. A pair of platinum stimulation electrodes was placed in the low right atrium. Hearts were loaded with a bolus injection (200 μL injected into the perfusate over 30 s, *i.e.* diluted in 20 mL of saline) of the voltage sensitive dye RH237 (Molecular Probes, OR USA) dissolved in DMSO (1 mg/mL). For optical mapping recordings, hearts were placed in a custom-built chamber which allowed control of bathing solution temperature and recording of global ECG *via* wall-fixed electrodes (Fig. 2A). The anterior surface of the heart was illuminated by 535 ± 25 nm light (interference filter, Comar Instruments Ltd, UK) from four 100 W tungsten-halogen lamps. Light emitted from the heart was collected using a camera lens (Nikon 85 mm, NA 1.4), passed through a 695 nm long-pass filter (Omega Optical Inc, USA) and focused onto a 16 × 16 photodiode array (C4675-102, Hamamatsu Photonics UK Ltd). Images were collected at 1 kHz from an area of 15 × 15 mm, saved to computer disk, and analyzed using custom software following application of a Gaussian spatial filter (radius 2 pixels) in accordance with the principles set out in Mironov et al. [25]. Artefacts in electric recordings, caused by motion, were reduced with 3 μmol/L cytochalasin-D (Sigma Aldrich, UK), which we previously found to have no significant effects on cardiac activation parameters in rabbits over the range of stimulus frequencies used in this study [26].

Isochronal maps of activation time were constructed, and conduction velocity was derived. The range of activation times in a given heart under specific pacing conditions was defined as the difference in timing between the earliest and latest action potential upstroke recorded by the photodiode array.

Transmural conduction through the LV free wall was assessed by pacing from the ventricular endocardium *via* a plunge bipolar electrode and monitoring earliest LV epicardial activation using optical mapping. LV longitudinal conduction velocity was assessed by pacing from the LV epicardium and monitoring optical signals at a 5 mm radius from the activation point.

2.4. Isolated AVN preparation functional studies

In a separate set of experiments, isolated AVN preparations (14 sham and 14 LVD) were prepared by the removal of all ventricular tissue, followed by an incision around the crest of the right atrial appendage which, when folded open, exposed the endocardial surface of the

Download English Version:

https://daneshyari.com/en/article/8473818

Download Persian Version:

https://daneshyari.com/article/8473818

Daneshyari.com