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Cardiac contractility modulation increases action potential duration dispersion and decreases ventricular fibrillation threshold via β1-adrenoceptor activation in the crystalloid perfused normal rabbit heart ☆☆

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ABSTRACT

Background/objectives: Cardiac contractility modulation (CCM) is a new treatment being developed for heart failure (HF) involving application of electrical current during the absolute refractory period. We have previously shown that CCM increases ventricular force through β1-adrenoceptor activation in the whole heart, a potential pro-arrhythmic mechanism. This study aimed to investigate the effect of CCM on ventricular fibrillation susceptibility.

Methods: Experiments were conducted in isolated New Zealand white rabbit hearts (2.0–2.5 kg, $n = 25$). The effects of CCM (±20 mA, 10 ms phase duration) on the left ventricular basal and apical monophasic action potential duration (MAPD) were assessed during constant pacing (200 bpm). Ventricular fibrillation threshold (VFT) was defined as the minimum current required to induce sustained VF with rapid pacing (30 × 30 ms). Protocols were repeated during perfusion of the β1-adrenoceptor antagonist metoprolol (1.8 μM). In separate hearts, the dynamic and spatial electrophysiological effects of CCM were assessed using optical mapping with di-4-ANEPPS.

Results: CCM significantly shortened MAPD close to the stimulation site (Basal: 102 ± 5 [CCM] vs. 131 ± 6 [Control] ms, $P < 0.001$). VFT was reduced during CCM (2.6 ± 0.6 [CCM] vs. 6.1 ± 0.8 [Control] mA, $P < 0.01$) and was correlated ($r^2 = 0.40$, $P < 0.01$) with increased MAPD dispersion (26 ± 4 [CCM] vs. 5 ± 1 [Control] ms, $P < 0.01$) ($n = 8$). Optical mapping revealed greater spread of CCM induced MAPD shortening during basal vs. apical stimulation. CCM effects were abolished by metoprolol and exogenous acetylcholine. No evidence for direct electrotonic modulation of APD was found, with APD adaptation occurring secondary to adrenergic stimulation.

Conclusions: CCM decreases VFT in a manner associated with increased MAPD dispersion in the crystalloid perfused normal rabbit heart.

1. Introduction

Cardiac contractility modulation (CCM) is an electrical device therapy being developed for the treatment of heart failure (HF), which remains a significant clinical burden despite decades of research and the development of useful drug therapy [1]. During CCM, electrical signals are applied to the ventricular myocardium to increase force and are timed to coincide with the absolute refractory period. Experimental and clinical studies demonstrate that CCM improves contractile performance, increases patient exercise capacity and improves quality of life (reviewed by Winter et al. [2]). Several studies have highlighted that CCM reverses the molecular remodelling associated with heart failure opposing the down-regulation of several calcium handling proteins (e.g. phospholamban, sarco-endoplasmic reticulum ATPase (SERCA)),

Abbreviations: ACh, acetylcholine; APD, action potential duration; CCM, cardiac contractility modulation; LV, left ventricle; $MAPD_{90}$, monophasic action potential duration at 90% repolarization; NE, norepinephrine; VFT, ventricular fibrillation threshold.

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increased expression of detrimental signalling factors (e.g. atrial natriuretic peptide) and fibrosis, which may contribute to its long term beneficial action [2,3].

We have previously demonstrated that the acute effects of CCM are mediated via stimulated norepinephrine (NE) release resulting in cardiac β1-adrenoceptor activation and shortening of locally recorded monophasic action potential duration (MAPD); in the crystalloid perfused normal rabbit heart [4]. The adrenergic dependence of CCM raises interesting questions regarding its neuro-cardiac action and the potential pro-arrhythmic nature of induced electrophysiological changes.

Efferent and afferent sympathetic and parasympathetic nerves richly innervate the cardiac ventricle and it has long been known that afferent nerves relay sensory information from the myocardium to the intracardiac and intra-thoracic ganglia, spinal cord and the brain for integration to regulate cardiac function [5]. It is recognised that local reflexes, acting through the cardiac and intra-thoracic ganglia, can modulate cardiac function on a beat-to-beat basis *without* involvement of the central nervous system [6]. It is perceivable that CCM captures both afferent and efferent nerve fibres, and that the former could engage intrathoracic ganglia or reflex activated sympathetic preganglionic neurones in the spinal cord. Furthermore, CCM may release acetylcholine (ACh) from parasympathetic nerves within the ventricle. ACh is known to antagonise NE synaptic release and antagonise post-synaptic adrenergic signalling and so it is necessary to establish whether ACh has a role in acute action of CCM (i.e. does ACh modulate the adrenergic component of CCM?).

It has long been acknowledged that adrenergic activation is pro-arrhythmic. We have previously shown that sympathetic nerve stimulation increases the susceptibility of the heart to ventricular fibrillation (VF) [7]. Given the adrenergic dependence of CCM and the pro-arrhythmic effect of positive inotropic drugs in HF, it is important to determine the effects of CCM on ventricular arrhythmia susceptibility [8].

The aims of this study were to investigate: 1) the role of pre and post-ganglionic signalling in CCM, 2) any role for ACh in modulating the acute ventricular response to CCM and 3) the effects of CCM on ventricular electrophysiology and arrhythmia susceptibility.

2. Methods

2.1. Animal welfare and ethical publishing declaration

Experiments were conducted on Adult male New Zealand White rabbit hearts (2.5–3.5 kg, $n = 22$), using the non-innervated and innervated heart preparations. All procedures were undertaken in accordance with ethical guidelines set out by the UK Animals (Scientific Procedures) Act 1986 and conformed 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 2010).

The author(s) of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology.

2.2. Isolation of the non-innervated heart preparation

Adult male New Zealand White rabbit hearts were isolated as previously described [4]. In brief, animals were pre-medicated with ketamine (Ketaset, 10 mg/kg, Fort Dodge, UK), medetomidine hydrochloride (Sedator, 0.2 mg/kg, Dechra, UK) and butorphanol (Torbugesic, 0.05 mg/kg, Fort Dodge, UK) (i.m.). Following stable sedation, animals were sacrificed with an overdose of pentobarbitone sodium (Sagatal, Rhone Merieux, UK; 111 mg/kg body weight, i.v.) containing Heparin (1000 IU, Multiparin, UK) delivered via the marginal ear vein. The hearts were rapidly excised, placed into ice cold tyrode solution to reduce metabolic rate and retrogradely perfused through the ascending aorta in conditions of constant flow Langendorff mode (40 ml/min) using a Gilson Minipuls 3 peristaltic pump (Anachem, UK).

2.3. Isolation of heart with intact autonomic innervation

The innervated Langendorff perfused isolated heart preparation was utilised as previously described [9]. An illustration of the preparation can be found in Fig. 1B. In brief, following pre-medication, anaesthesia was maintained with i.v. propofol (5 mg as required, Rapinovet, Schering-Plough Animal Health, UK) for the remainder of surgery. Following tracheotomy animals were ventilated with room air using a small animal ventilator (Harvard Apparatus Ltd, UK; 60breaths/min). The left and right vagus nerves were isolated, and the blood vessels leading to and from the ribcage were ligated and dissected. Animals were sacrificed with an overdose of pentobarbitone sodium containing heparin (as above). The anterior portion of the ribcage was removed, and the descending aorta was cannulated. The pericardium was cut, and ice-cold Tyrode solution was applied to the surface of the heart. The preparation, extending from C1 to T12 vertebrae, was dissected from surrounding tissues and perfused through the descending aorta (100 ml/min) [9].

2.4. Solutions

Hearts were perfused with Tyrode solution of the following composition (mM): Na^+ 138.0, K^+ 4.0, Ca^{2+} 1.8, Mg^+ 1.0, HCO_3^- 24.0, $H_2PO_4^-$ 0.4, Cl^- 121.0, glucose 11.0 and acetate 20.0. The solution was continuously bubbled with 95% O_2–5% CO_2 to maintain a constant pH of 7.4. Temperature was maintained at 37 °C. A 3 F polypropylene catheter (Portex, UK) was inserted at the left ventricular (LV) apex for drainage of Thebesian venous effluent.

2.5. Functional parameters

Intra-ventricular LV pressure (LVP) was measured with a fluid-filled latex balloon connected to a pressure transducer (MTL0380, ADInstruments Ltd, UK) inserted into the LV via the left atrium. LV end diastolic pressure was maintained between 0 and 5 mm Hg. Aortic perfusion pressure (PP) was monitored with a second pressure transducer connected in series to the aortic cannula.

2.6. Cardiac electrical recording and pacing

MAP-TIP recording catheters (73-0150, Harvard Apparatus, UK), with a tip diameter of 1.5 mm electrode spacing of 4 mm, were used to record action potentials from the epicardial surface of the LV using a DC-coupled high input impedance differential amplifier (Joint Biomedical Workshop, University of Leicester, UK). MAPs were recorded from the LV epicardium at apical and/or basal sites depending on the experimental protocol.

Pacing electrode locations are illustrated on Fig. 1A. Endocardial pacing electrodes were inserted through the pulmonary trunk or left atrial appendage for RV and LV apical endocardial pacing, respectively (electrode diameter = 2 mm, spacing = 4 mm). For epicardial pacing, a pair of platinum hook electrodes (spacing = 4 mm) were inserted into the basal anterior LV freewall. Pacing stimuli delivered using a constant current electrical stimulator (DS7A, Digitimer Ltd, UK).

2.7. CCM signal generation and delivery

Square wave electrical pulses were generated using a Neurolog modular system (Digitimer, UK) with signals delivered using a constant current stimulator (Model A385, World Precision Instruments, UK). CCM stimuli were triggered using the pacing stimulus as previously described [4]. CCM signal were applied to the epicardial surface of the LV through a pair of platinum hook electrodes (inter-electrode distance ~ 1 cm) as a biphasic waveform with equal positive and negative phase amplitudes (stimulus amplitude = 20 mA and phase duration = 10 ms). We have previously demonstrated that these parameters induce optimal responses [4]. CCM signals were timed to coincide with the plateau phase using a locally recorded MAP (see Fig. 1.) and were delivered 2–3 min, allowing for a 30 second period of steady state. A 10–15 minute rest period between stimulations was used throughout the experiment for parameters to return to baseline. Hearts were paced during all CCM protocols at a 300 ms cycle length.

2.7.1. Protocols

2.7.1.1. Role of the intracardiac/intrathoracic ganglia. The effects of CCM on ventricular and electrophysiological performance were assessed in the *innervated Langendorff heart* in the absence and presence of the nicotinic ACh channel antagonist hexamethonium (0.5 mM) [10]. Ganglionic blockade was confirmed by inhibition of bradycardia with right vagus nerve stimulation (5Hz, 5 V). CCM was applied in basal regions of the LV.

2.7.1.2. Role of ACh. The effects of CCM on ventricular and electrophysiological performance were assessed in *the non-innervated Langendorff heart* preparation in the absence (Control) and presence of;

A. The muscarinic ACh receptor antagonist atropine (0.1 mM) [10].
B. Exogenous ACh (1 μM) [13] with and without atropine (0.1 μM) [11].

2–3 stimulations were conducted at baseline and during perfusion. The order of perfusion was randomised from heart to heart to reduce any bias. CCM was applied in basal regions of the LV.

2.7.1.3. VFT, ERP and APD dispersion

2.7.1.3.1. VFT. The susceptibility of the heart to VF was studied by measuring the ventricular fibrillation threshold (VFT) in the *non-innervated Langendorff heart*. VFT was obtained by RV endocardial, LV endocardial or LV epicardia pacing using a train of 30-stimuli (CL = 30 ms, 2 ms pulse width) spanning the refractory period following a 20-beat S1 drive train (CL = 300 ms, 2 ms pulse width) and determined by progressively increasing the pacing current (0.5 mA steps), with 2-second rest period before the next pacing train if no VF was induced (see Fig. 2). VFT was defined as the minimum current required to induce sustained VF. VFT was assessed in the absence and