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Original article

Selective heart rate reduction with ivabradine slows ischaemia-induced electrophysiological changes and reduces ischaemia–reperfusion-induced ventricular arrhythmias

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ABSTRACT

Heart rates during ischaemia and reperfusion are possible determinants of reperfusion arrhythmias. We used ivabradine, a selective I_f current inhibitor, to assess the effects of heart rate reduction (HRR) during ischaemia–reperfusion on reperfusion ventricular arrhythmias and assessed potential anti-arrhythmic mechanisms by optical mapping. Five groups of rat hearts were subjected to regional ischaemia by left anterior descending artery occlusion for 8 min followed by 10 min of reperfusion: (1) Control n = 10; (2) 1 μM of ivabradine perfusion n = 10; (3) 1 μM of ivabradine + 5 Hz atrial pacing throughout ischaemia–reperfusion n = 5; (4) 1 μM of ivabradine + 5 Hz pacing only at reperfusion; (5) 100 μM of ivabradine was used as a 1 ml bolus upon reperfusion. For optical mapping, 10 hearts (ivabradine n = 5; 5 Hz pacing n = 5) were subjected to global ischaemia whilst transmembrane voltage transients were recorded. Epicardial activation was mapped, and the rate of development of ischaemia-induced electrophysiological changes was assessed. HRR observed in the ivabradine group during both ischaemia (195 ± 11 bpm vs. control 272 ± 14 bpm, $p<0.05$) and at reperfusion (168 ± 13 bpm vs. 276 ± 14 bpm, $p<0.05$) was associated with reduced reperfusion ventricular fibrillation (VF) incidence (20% vs. 90%, $p<0.05$). Pacing throughout ischaemia–reperfusion abolished the protective effects of ivabradine (100% VF), whereas pacing at reperfusion only partially attenuated this effect (40% VF). Ivabradine, given as a bolus at reperfusion, did not significantly affect VF incidence (80% VF). Optical mapping experiments showed a delay to ischaemia-induced conduction slowing (time to 50% conduction slowing: 10.2 ± 1.3 min vs. 5.1 ± 0.7 min, $p<0.05$) and to loss of electrical excitability in ivabradine-perfused hearts (27.7 ± 4.3 min vs. 14.5 ± 0.6 min, $p<0.05$). Ivabradine administered throughout ischaemia and reperfusion reduced reperfusion VF incidence through HRR. Heart rate during ischaemia is a major determinant of reperfusion arrhythmias. Heart rate at reperfusion alone was not a determinant of reperfusion VF, as neither a bolus of ivabradine nor pacing immediately prior to reperfusion significantly altered reperfusion VF incidence. This anti-arrhythmic effect of heart rate reduction during ischaemia may reflect slower development of ischaemia-induced electrophysiological changes.

1. Introduction

Ventricular arrhythmias, can occur within seconds of restoration of blood flow to previously ischaemic myocardium, as initially shown by Tennant and Wiggers just under a century ago [1]. Reperfusion after brief periods of ischaemia, lasting seconds to minutes, occurs in the context of coronary artery vasospasm, or unstable angina, and is associated with reperfusion ventricular arrhythmias [2]. Reperfusion after longer periods of ischaemia lasting several hours occurs during percutaneous coronary intervention for patients with acute myocardial infarction, which is also associated with reperfusion arrhythmias [3].

The arrhythmogenic mechanisms that underlie reperfusion ventricular tachycardia (VT) and ventricular fibrillation (VF) are thought to relate to the abrupt electrophysiological and biochemical changes brought about by the restoration of blood flow [4]. The reperfusion of ischaemic myocardium triggers the rapid and heterogeneous restoration of action potentials towards that of pre-ischaemic levels [5], and the marked spatial heterogeneity in action potentials immediately after reperfusion can predispose to re-entry [6]. Reperfusion can also lead to intracellular calcium overloading [7], which then increases the electrogenic forward mode activity of the sarcolemmal sodium–calcium exchanger, and can cause Phase 4 delayed after depolarisations and triggered action potentials.

It has been shown in experimental studies that heart rate during acute ischaemia–reperfusion is an important determinant of

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susceptibility to reperfusion arrhythmias [8–10], with higher heart rates predisposing to arrhythmias. These data suggest that a clinical therapeutic strategy of heart rate reduction during ischaemia–reperfusion may reduce the incidence of reperfusion arrhythmias. However, it remains unclear if the heart rate during ischaemia or during reperfusion is more important in determining reperfusion arrhythmia susceptibility, and conflicting experimental evidence exists in support of either hypothesis [8,11].

Ivabradine (IVA) is a selective I_f current blocker with heart rate lowering effects [12], clinically licensed for chronic stable angina and chronic heart failure. Ivabradine treatment has also been shown to reduce the incidence of cardiovascular death and hospital admissions in a heart failure population in the SHIFT study [13], and it reduced the number of coronary events in a subgroup of chronic stable angina patients with heart rates of 70 beats per minute or greater in the BEAUTIFUL study [14,15]. Based on preclinical data showing that reduced heart rates beneficially impacted on reperfusion arrhythmia risk [8–11], we hypothesised that ivabradine treatment may provide anti-arrhythmic protection against reperfusion arrhythmias.

We assessed if heart rate lowering with ivabradine can reduce the incidence of reperfusion arrhythmias in an ex vivo rat model of acute regional ischaemia–reperfusion. We also sought to clarify if the heart rate during ischaemia or during reperfusion was more important in determining reperfusion arrhythmia susceptibility, as this will influence the timing of ivabradine administration in any potential clinical therapeutic strategy. We performed studies using ivabradine and atrial pacing in varying combinations to address these questions. We then performed optical mapping of transmembrane voltage to investigate the mechanism by which heart rate may influence reperfusion arrhythmogenesis.

2. Materials and methods

2.1. Ethical approval

This work was performed in accordance with standards set out in the UK Animals (Scientific Procedures) Act 1986.

2.2. Acute ischaemia–reperfusion studies

Thirty-five adult male Sprague–Dawley rats (weight 250–300 g) were sacrificed, and hearts were rapidly excised and retrogradely perfused via the aorta with modified Krebs–Henseleit solution (in mmol/l: NaCl 118.5, $CaCl_2$ 1.85, KCl 4.5, glucose 11.1, $NaHCO_3$ 25, $MgSO_4$ 2.5, NaH_2PO4 1.4) gassed with 95% O_2/5% CO_2 at 37 °C ± 0.5 °C and pH 7.35 ± 0.05. Perfusion pressure through the aorta was constant and maintained between 90 and 100 cm H_2O. Unipolar electrograms were recorded at a sampling frequency of 1 kHz using a silver electrode placed at the left ventricular anterolateral wall and a reference electrode attached to the aortic cannula. Electrodes were connected to a Bioamplifier and a PowerLab data acquisition system (AD Instruments, Sydney, Australia). During the stabilisation phase, a 6.0 Ethicon prolene ligature (Johnson & Johnson Ethicon, Livingston, UK) was placed around the left anterior descending (LAD) artery 1–2 mm distal from where it emerges beneath the left atrium, initially without tension (Fig. 1A).

The hearts were stabilised for 15 min before being randomly allocated to one of five treatment groups (Fig. 2D). Atrial pacing and ivabradine (IVA) were used to control heart rates during the experiments. Right atrial pacing was used and ventricular pacing avoided as ventricular pacing per se has been shown to facilitate the development of reperfusion arrhythmias [16]. An ivabradine concentration of 1 μM was selected for the acute ischaemia–reperfusion experiments based on concentration–response studies showing an 18 ± 4% reduction in sinus heart rate with this concentration (see Figs. 2A–C and Data supplement), a similar magnitude to the clinical heart rate reduction (HRR) reported in the SHIFT trial (~16% HRR) [13]. Ivabradine was kindly provided by Servier Laboratories, France.

Group 1 — Control (n = 10): Hearts were subjected to 8 min of ischaemia followed by 10 min of reperfusion, without pacing or ivabradine.

Group 2 — 1 μM of ivabradine (IVA, n = 10): Hearts were perfused with 1 μM of ivabradine, starting 5 min before ischaemia onset and then throughout the experiment. This group had reduced heart rates at ischaemia onset, throughout the duration of ischaemia and at reperfusion.

Group 3 — Ivabradine bolus at reperfusion (n = 5): Hearts were perfused with a 1 ml bolus of 100 μM of ivabradine 1 min prior to reperfusion. This group had reduced heart rates at reperfusion only.

Group 4 — 1 μM of ivabradine + atrial pacing during ischaemia and reperfusion (n = 5): Hearts were perfused with 1 μM of ivabradine, starting 5 min before ischaemia onset and throughout the experiment, matching Group 2. In addition, hearts were paced from the right atrium at 300 beats per minute (bpm) from the onset of ischaemia until the end of the experiment.

Group 5 — 1 μM of ivabradine + atrial pacing across reperfusion (n = 5): Hearts were perfused with 1 μM of ivabradine, starting 5 min before ischaemia onset and throughout the experiment, matching Groups 2 and 4, and were paced at 300 bpm starting 30 s before reperfusion and for a further minute after onset of reperfusion.

To generate regional ischaemia, the prolene ligature placed earlier around the LAD artery was tightened using a polythene occluder tube placed over the suture, thereby occluding flow through the artery (Fig. 1A). Successful occlusion of the artery was confirmed by a reduction in coronary flow rate (CFR) of >30% as assessed by measuring the coronary effluent, in conjunction with an alteration in the axis and morphology of the unipolar electrogram. Hearts were subjected to 8 min of regional ischaemia, before the suture was released to restore flow across the LAD artery. We selected 8 min of ischaemia because the relationship between ischaemia duration and reperfusion VT/VF incidence is "bell-shaped" [8], with our data showing that 8 min of ischaemia is on the steep part of this bell-shaped relationship (see Data supplement). Reperfusion was confirmed by an increase in CFR towards that of pre-ischaemic values. The reperfusion phase was continued for 10 min with continuous electrogram monitoring for all 5 groups. The incidence of ventricular arrhythmias during the ischaemia and reperfusion were recorded. Ventricular arrhythmias were classified according to the Lambeth Convention guidelines [17]. The primary endpoints were reperfusion VT incidence and reperfusion VF incidence.

2.3. Optical mapping studies during global no-flow ischaemia

In order to assess the effects of heart rate on the rate of development of ischaemia-induced electrophysiological changes, optical mapping experiments (Fig. 1B) were performed in a global no-flow ischaemia model. Ten hearts were perfused at fixed coronary flow rates (15 ml/min) inside a perspex optical mapping chamber (Cairn Research, Faversham, UK) (Fig. 1C). Following a 15-minute stabilisation period, the hearts were stained with RH237, a potentiometric dye (25 μl of 1 mg/ml RH237 in dimethyl sulfoxide; Invitrogen, UK), and perfused with an excitation–contraction uncoupler, 10 μM of blebbistatin (Sigma-Aldrich, UK), to eliminate motion artefact [18].

To record optical action potentials, the hearts were excited with light-emitting diodes (excitation wavelength 530 nm), and the emitted fluorescent light was collected and split with a dichroic mirror at 630 nm. The shorter wavelength light portion was focused onto a