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Original Article

Reduced response to I_{Kr} blockade and altered hERG1a/1b stoichiometry in human heart failure

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ABSTRACT

Heart failure (HF) claims 250,000 lives per year in the US, and nearly half of these deaths are sudden and presumably due to ventricular tachyarrhythmias. QT interval and action potential (AP) prolongation are hallmark proarrhythmic changes in the failing myocardium, which potentially result from alterations in repolarizing potassium currents. Thus, we aimed to examine whether decreased expression of the rapid delayed rectifier potassium current, I_{Kr}, contributes to repolarization abnormalities in human HF. To map functional I_{Kr} expression across the left ventricle (LV), we optically imaged coronary-perfused LV free wall from donor and end-stage failing human hearts. The LV wedge preparation was used to examine transmural AP durations at 80% repolarization (APD80), and treatment with the I_{Kr}-blocking drug, E-4031, was utilized to interrogate functional expression. We assessed the percent change in APD80 post-I_{Kr} blockade relative to baseline APD80 (ΔAPD80) and found that ΔAPD80s are reduced in failing versus donor hearts in each transmural region, with 0.35-, 0.43-, and 0.41-fold reductions in endo-, mid-, and epicardium, respectively ($p = 0.008$, 0.037, and 0.022). We then assessed hERG1 isoform gene and protein expression levels using qPCR and Western blot. While we did not observe differences in *hERG1a* or *hERG1b* gene expression between donor and failing hearts, we found a shift in the hERG1a:hERG1b isoform stoichiometry at the protein level. Computer simulations were then conducted to assess I_{Kr} block under E-4031 influence in failing and nonfailing conditions. Our results confirmed the experimental observations and E-4031-induced relative APD80 prolongation was greater in normal conditions than in failing conditions, provided that the cellular model of HF included a significant downregulation of I_{Kr}. In human HF, the response to I_{Kr} blockade is reduced, suggesting decreased functional I_{Kr} expression. This attenuated functional response is associated with altered hERG1a:hERG1b protein stoichiometry in the failing human LV, and failing cardiomyoctye simulations support the experimental findings. Thus, of I_{Kr} protein and functional expression may be important determinants of repolarization remodeling in the failing human LV.

1. Introduction

Heart failure (HF) is the end-stage of many cardiovascular diseases, in which the heart can no longer support the metabolic demands of the body. HF is an increasing problem in the US, with an estimated 5.8 million Americans currently afflicted by the disease [1,2]. Approximately ¼ million HF-related deaths occur annually, nearly half of which are due to sudden cardiac death [3]. These sudden cardiac events are presumably the result of ventricular tachyarrhythmias, which are a consequence of adverse electrophysiologic remodeling during the HF progression.

Action potential (AP) prolongation and resulting QT prolongation are hallmark arrhythmogenic changes in the failing myocardium [4–7]. While increased late sodium current has been demonstrated in association with AP prolongation in HF [8–10], voltage-dependent potassium currents are critical determinants of cardiac AP duration (APD) [11]. In humans, the rapid component of the delayed rectifier potassium current (I_{Kr}) is largely responsible for ventricular repolarization. Tetramers of the hERG1 protein α-subunit, encoded by the *KCNH2* gene, form the channel underlying cardiac I_{Kr}. Two different splice variants of *KCNH2*, both *hERG1a* and *hERG1b*, are expressed in human ventricular tissue, with the *hERG1a* isoform predominating [12]. In various animal models of HF, delayed rectifier potassium currents are reduced [13,14]. However, in human isolated cardiomyocytes, I_{Kr} amplitude is small, making differences between donor and failing hearts undetectable [4].

Abbreviations: AP, action potential; APD, action potential duration; APD80, action potential duration at 80% repolarization; D, donor; Endo, endocardium; Epi, epicardium; HF, heart failure; F, failing; LV, left ventricle; Mid, midmyocardium.

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We hypothesized that I_{Kr} is downregulated in human HF, promoting repolarization abnormalities in failing myocardial tissue. Thus, we aimed to investigate functional I_{Kr} expression in the failing human left ventricle (LV) and examine the relative expression of hERG1a and hERG1b isoforms at the gene and protein expression levels. We then conducted cellular and fiber simulation studies to provide further support for I_{Kr} downregulation in HF. The regulation of functional I_{Kr} in human HF has not been previously reported; thus, these studies will help elucidate the underpinnings of repolarization remodeling in the failing human heart.

2. Materials and methods

2.1. Human heart recovery

All studies using human heart tissue have been approved by the Institutional Review Board at Washington University in St. Louis. In total for this study, we recovered 16 donor human hearts, rejected for transplantation from the Mid America Transplant Services (St. Louis, MO), and 14 end-stage failing hearts from transplant recipients at Barnes-Jewish Hospital. All hearts were obtained immediately after removal from the chest in the operating room. Hearts were arrested using ice-cold cardioplegic solution and transported to the laboratory for dissection and functional experiments. Prior to experiments, LV tissue was collected and preserved in RNA later (Sigma-Aldrich, St. Louis, MO) for mRNA or flash-frozen in liquid nitrogen for protein expression analyses.

2.2. Optical imaging

Human LV wedge preparations were used for electrophysiologic experiments, as described previously [5]. Briefly, wedges were dissected from an LV marginal branch and were mounted with the transmural surface facing the optical apparatus (Fig. 1A–B). Preparations were perfused with oxygenated Tyrode's solution maintained at 37 °C, with a perfusion pressure of 60–80 mmHg. Blebbistatin (10–20 μM) was used to immobilize myocardial tissue, and Di-4-ANEPPS was used to map transmembrane potential. Pseudo-ECGs were recorded with Ag/AgCl electrodes placed on either side of the transmural surface, and human intracellular APDs were validated using fixed 3.0 M KCl filled microelectrodes. Tissue was paced using a steady-state S1S1 restitution protocol, starting at a pacing cycle length (CL) of 2000 ms and progressively decreasing to the functional refractory period. Data were analyzed using custom-written MATLAB software [15]. Table 1 shows donor and patient characteristics of hearts used in functional experiments.

2.3. Pharmacologic interrogation of I_{Kr}

Following the collection of baseline restitution measurements, we added 1 μM E-4031, a high-affinity I_{Kr} blocker, to the Tyrode's solution. Recordings were collected at 5-minute intervals after drug treatment, until a steady-state AP morphology was achieved (approximately 15–20 min). The steady-state restitution protocol was then repeated (Fig. 1C). Because E-4031 blockade of I_{Kr} is essentially irreversible, we did not conduct drug washout.

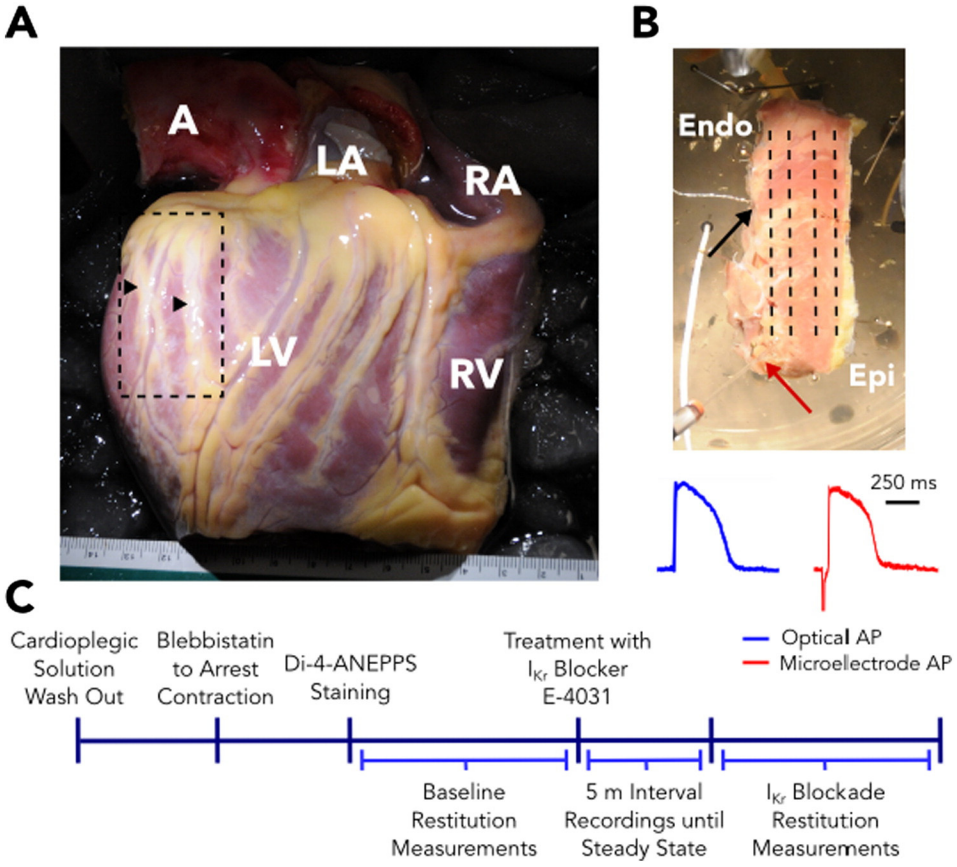

Fig. 1. Experimental methodology. A. Posterolateral image of a human heart. Black dashed box outlines marginal artery territory for wedge preparation, and black arrowheads indicate two descending marginal arteries. B. Representative wedge preparation image with paired optical (blue) and microelectrode (red) recordings. Wedge transmural regions separated by dashed lines, and black and red arrows highlight pacing and microelectrodes, respectively. C. Timeline of the experimental protocol. A = aorta; Epi = epicardium; Endo = endocardium; LA = left atrium; LV = left ventricle; RA = right atrium; RV = right ventricle. For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.

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