Cardiac activation–repolarization patterns and ion channel expression mapping in intact isolated normal human hearts

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BACKGROUND The repolarization pattern of the human heart is unknown.

OBJECTIVE The purpose of this study was to perform a multisite analysis of the activation–repolarization patterns and mRNA expression patterns of ion channel subunits in isolated human hearts.

METHODS Hearts from 3 donors without reported cardiac disease were Langendorff perfused with the patient's own blood. A standard ECG was obtained before explantation. Up to 92 unipolar electrograms from 24 transmural needles were obtained during right atrial pacing. Local activation and repolarization times and activation–recovery intervals (ARI) were measured. The mRNA levels of subunits of the channels carrying the transient outward current and slow and rapid components of the delayed rectifier current were determined by quantitative reverse transcriptase polymerase chain reaction at up to 63 sites.

RESULTS The repolarization gradients in the 3 hearts were different and occurred along all axes without midmural late repolarization. A negative activation–repolarization relationship occurred along the epicardium, but this relationship was positive in the whole hearts. Coefficients of variation of mRNA levels (40%–80%) and of the Kv7.1 protein (alpha-subunit slow delayed rectifier channel) were larger than those of ARIs (7%–17%). The regional mRNA expression patterns were similar in the 3 hearts, unlike the ARI profiles. The expression level of individual mRNAs and of Kv7.1 did not correlate with local ARIs at the same sites.

CONCLUSION In the normal human heart, repolarization gradients encompass all axes, without late midmural repolarization. Last activated areas do not repolarize first as previously assumed. Gradients of mRNAs of single ion channel subunits and of ARIs do not correlate.

KEYWORDS Human heart; Repolarization pattern; Activation–recovery interval; Activation pattern; mRNA expression levels; Kv7.1 protein; Cardiac ion channel

Introduction

The ventricular repolarization sequence determines the morphology of the T wave.[1] Dispersion of repolarization plays a major role in the genesis of ventricular arrhythmias.[2,3] Despite its clinical relevance, the repolarization sequence of the human heart is essentially unknown. Only a few studies have explored the local repolarization times (RTs) in diseased hearts, and these recordings were limited to the endocardium or epicardium.[4–7] The local RT is the sum of the local activation time (AT) and the duration of the local action potential. The latter can be indirectly assessed by the duration of the activation-recovery intervals (ARIs) from local electrograms.[8]

Current cardiology textbooks sustain the assumption that the last areas to be activated repolarize first.[9] Therefore, a negative relationship between activation and RTs is presumed. However, Hanson et al[10] have shown that in patients undergoing cardiac surgery, the slope of the AT–RT

This study has been supported by a grant from Spanish Fundació Marató TV3 Project 080630 to Drs. Coronel and Cinca, and Arlin Tasiam; and by a ZonMW/NWO Innovational Research Incentives Scheme VIDI Grant to Dr. Remme. Drs. Coronel and Cinca contributed equally to this manuscript.
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http://dx.doi.org/10.1016/j.hrthm.2016.10.010

relationship is positive along the endocardium. In canine hearts, the AT–RT relationship also is positive.[11]

The intrinsic duration of the action potential is determined by the balance between inward and outward currents flowing during the plateau and repolarization phases. Thus, it can be anticipated that regional differences in expression of genes encoding for repolarizing channels will lead to different repolarization patterns.

This study was undertaken to assess the sequence and distribution of cardiac repolarization in relation to the activation process and local RNA expression of the ion channels carrying the major repolarizing currents in isolated perfused normal human hearts. The latter was quantitatively determined at the same locations where local electrograms were obtained, allowing direct correlation with ARI. The density of the alpha-subunit of the slow component of the delayed rectifier channel was measured as well. Our study does not permit a direct comparison between repolarization patterns and the T wave on the surface ECG.

Methods

Study material

We analyzed 3 human hearts obtained from organ donors with no reported cardiac disease but unsuitable for cardiac transplantation. These patients died because of irreversible cerebrovascular lesions at the Hospital de la Santa Creu i Sant Pau in Barcelona, Spain. Written informed consent for explantation had been obtained from the relatives. The hearts were extracted and immediately processed for experimental studies. The investigation conforms to the principles of the Declaration of Helsinki and was approved by the ethics review board of the hospital. Clinical variables, laboratory data, and conventional 12-lead ECG were obtained on admission and before cardiac explantation.

Electrical mapping and data analysis

The explanted hearts were Langendorff-perfused at 37°C and stimulated from the right atrium at cycle length of 700 ms. After an equilibration period of 30–60 minutes, local unipolar electrograms were simultaneously recorded (duration of each registration 2 seconds) from up to 24 transmural needles inserted in the left ventricle (LV) and septum (needles harboring 4 electrodes at 4-mm distance) and in the right ventricular (RV) (needles with 3 electrodes at 4-mm distance). In each electrogram, activation time (AT) and RTs were detected automatically as the moment of dV/dt_{min} during the QRS complex and the moment of dV/dt_{max} during the T wave (relative to the earliest start of the QRS complex in any of the electrodes),[8,12,13] respectively, as shown in Online Supplementary Figure 1. Subtraction of AT from RT yielded ARI. Activation, repolarization, and ARI patterns were manually constructed. To allow comparison with the molecular biologic data, ARI data were also expressed as a fraction of the average value of the entire heart (see Online Supplemental Material for details).

Molecular analysis

Myocardial samples were obtained from each heart adjacent to the sites with electrophysiologic data. Transmural samples were divided into equal epicardial, mid, and endocardial portions, except in the RV myocardium, which were divided into equal epicardial and endocardial portions. Each sample was immediately snap-frozen in liquid nitrogen and stored at –80°C. A maximum of 63 samples (18 RV, 18 septum, 27 LV) was obtained per heart.

mRNA transcript analysis

Quantitative polymerase chain reaction data of mRNAs encoding for alpha- and beta-units of the channels carrying I_{to}, I_{Ks}, and I_{Kr} were analyzed with the LinRegPCR program.[14] Samples were measured in duplicate and normalized to the housekeeping gene *HPRT*. Regional RNA expression is provided as a fraction relative to the average of the respective mRNA in the whole heart. Details of the isolation and sequences of primers are given in Online Supplementary Table 1.

Western blot analysis

For western blotting, 15 μg of total protein (whole cell lysate isolated from human heart tissue samples) was separated on a 4%–20% gradient SDS-PAGE gel and incubated with the following primary antibodies: anti-GAPDH (1:1000; catalog no. 10R-G109a, Fitzgerald Industries International, Acton, MA, USA) and anti-Kv7.1 (KCNQ1m 1:200; catalog no. APC-022, Alomone Labs, Jerusalem, Israel). All samples were blotted in triplicate, and results were represented as KCNQ1 density relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Details on protein isolation, blotting procedure, quantification, and data analysis are provided in the Online Supplemental Material.

Statistical analysis

Analysis of variance was used to test regional differences of local electrophysiologic variables. mRNA expression levels between regions of the heart were tested nonparametrically with the Kruskal–Wallis test. Post hoc analysis was performed by either the Student–Newman–Keuls test or by the Dunn test. AT–RT, mRNA-ARI, protein–ARI, and mRNA–protein relationships (all at the same sites) were analyzed using linear regression analysis. $P < .05$ was considered significant.

Results

Clinical findings

Table 1 summarizes the clinical, ECG, and echocardiographic data of the 3 donors. Online Supplementary Figure 2 shows normal T waves in donor 1 but negative T waves in donors 2 and 3 at the time of cardiac explantation. In the case of donor 2, the negative T waves are asymmetric and appear in leads V_4–V_6, whereas in donor 3 the negative T waves are symmetric and altered in all precordial leads. Donor 2 had a previous history of arterial hypertension, and the echocardiogram suggests mild-to-moderate LV hypertrophy.