From beat rate variability in induced pluripotent stem cell–derived pacemaker cells to heart rate variability in human subjects

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BACKGROUND We previously reported that induced pluripotent stem cell–derived cardiomyocytes manifest beat rate variability (BRV) resembling heart rate variability (HRV) in the human sinoatrial node. We now hypothesized the BRV-HRV continuum originates in pacemaker cells.

OBJECTIVE To investigate whether cellular BRV is a source of HRV dynamics, we hypothesized 3 levels of interaction among different cardiomyocyte entities: (1) single pacemaker cells, (2) networks of electrically coupled pacemaker cells, and (3) the *in situ* sinoatrial node.

METHODS We measured BRV/HRV properties in single pacemaker cells, induced pluripotent stem cell–derived contracting embryoid bodies (EBs), and electrocardiograms from the same individual.

RESULTS Pronounced BRV/HRV was present at all 3 levels. The coefficient of variance of interbeat intervals and Poincaré plot indices SD1 and SD2 for single cells were 20 times greater than those for EBs ($P < .05$) and the *in situ* heart (the latter two were similar; $P > .05$). We also compared BRV magnitude among single cells, small EBs (~5–10 cells), and larger EBs (>10 cells): BRV indices progressively increased with the decrease in the cell number ($P < .05$). Disrupting intracellular Ca^{2+} handling markedly augmented BRV magnitude, revealing a unique bimodal firing pattern, suggesting that intracellular mechanisms contribute to BRV/HRV and the fractal behavior of heart rhythm.

CONCLUSION The decreased BRV magnitude in transitioning from the single cell to the EB suggests that the HRV of *in situ* hearts originates from the summation and integration of multiple cell-based oscillators. Hence, complex interactions among multiple pacemaker cells and intracellular Ca^{2+} handling determine HRV in humans and cardiomyocyte networks.

KEYWORDS Electrophysiology; Heart rate; Induced pluripotent stem cells; Heart rate variability; Cardiac myocytes

ABBREVIATIONS **BRV** = beat rate variability; **DFA** = detrended fluctuation analysis; **EB** = embryoid body; **ECG** = electrocardiogram; **HRV** = heart rate variability; **IBI** = interbeat interval; **IBI COV** = interbeat interval coefficient of variance; $\mathbf{I_f}$ = The funny current; **iPSC** = induced pluripotent stem cell; **iPSC-CM** = induced pluripotent stem cell–derived cardiomyocyte; **SAN** = sinoatrial node; **SR** = sarcoplasmic reticulum; **SSS** = sick sinus syndrome

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Introduction

Numerous studies have shown that the normal heart rate fluctuates around a mean, a phenomenon termed heart rate variability (HRV).[1,2] A key feature of the human heart is that HRV exhibits self-similar fractal-like oscillations contributing to its complex firing pattern.[3,4] The common notion is that HRV reflects the opposing effects of the autonomic nervous system branches as well as other extra- and intracardiac inputs, for example, intracellular, chemical, hormonal and metabolic. Yet, HRV persists in denervated hearts (even though differently than innervated hearts), suggesting that it derives from other extracardiac factors affecting heart rate dynamics as well as from intrinsic cardiac regulatory mechanisms.[5]

We recently reported that networks (clusters) of human embryonic stem cell–derived cardiomyocytes and induced pluripotent stem cell (iPSC)–derived cardiomyocytes (iPSC-CMs) completely lacking extracardiac inputs exhibit beat rate variability (BRV), fractality, and power-law behavior

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resembling HRV in the human sinoatrial node (SAN).[4,6] This result was consistent with the studies on ventricular cells of newborn rats (both isolated cells and cell networks), which also found fractal and power-law behavior.[7,8] Collectively, our findings suggest that these BRV properties are intrinsic to pacemaker cells. A key question arising from the resemblance of BRV in cardiomyocyte-coupled networks to HRV *in situ* is whether BRV is a fundamental source of HRV. Hence, the sources of HRV *in situ* can be considered in terms of non–steady-state firing patterns of individual cardiac pacemaker cells, interactions among neighboring pacemaker cells in a coupled network, and extracardiac effects on these components. To investigate whether cellular BRV is a source of HRV dynamics, we hypothesized 3 levels of interaction among different cardiomyocyte entities: (1) single pacemaker cells, (2) networks of electrically coupled pacemaker cells, and (3) the *in situ* SAN. To test this hypothesis, we analyzed HRV in electrocardiograms (ECGs) of healthy volunteers and BRV in extracellular electrograms and action potentials recorded from spontaneously contracting embryoid bodies (EBs) and iPSC-CM, respectively, both fabricated from volunteers' keratinocytes. We also hypothesized that disrupting intracellular Ca^{2+} handling would affect the BRV magnitude. If validated, this would suggest that intracellular mechanisms contribute to BRV/HRV and the fractal behavior of cardiac rhythm.

Methods

ECGs were recorded from 5 healthy female individuals (aged 25–54 years) taking no medication. We plucked 10 scalp hairs per subject from 2 of them and prepared iPSC-CMs from keratinocytes as described previously[9] (see the Online Supplement). The study was approved by the local ethics committee, and all subjects gave informed consent. Our previous article reported a normal karyotype and electrographic properties recorded from EBs generated from 1 volunteer (#201201) included in the present work.[6,9] iPSCs and iPSC-CMs from the second volunteer (#201202) included in this study have a normal karyotype and comparable electrophysiological data (data not shown).

Data recording and processing

Extracellular electrograms were recorded for 30 minutes from spontaneously contracting 18–39-day-old EBs by using the microelectrode array apparatus (Multi Channels Systems, Reutlingen, Germany).[6] Recordings were done at a sampling frequency of 1000 Hz, downsampled to 200 Hz, and analyzed to detect peaks of the signal from which R-R intervals were calculated by using the MATLAB software (Mathworks, Massachusetts). Action potentials were recorded from single cardiomyocytes or small EBs containing few cells, as described below. ECGs were recorded from 5 healthy individuals and analyzed as described in the Online Supplement.

Action potential recordings

For action potential recordings, spontaneously contracting areas of EBs were mechanically dissociated and enzymatically dispersed (collagenase type II 1 mg/mL; Worthington, Lakewood, NJ, http://www.worthington-biochem.com). This dispersion resulted in single cells, small contracting clusters (5–10 cells), and larger contracting clusters (>10 cells). Single cardiomyocytes as well as small and large clusters were then plated on gelatin-coated glass coverslips (13 mm in diameter) in 24-well plates. The coverslips were incubated at 37°C, and a recovery period of 2 days was allowed before the electrophysiological experiment was performed.[10] In all experiments, the coverslips were perfused at 37°C with an external solution containing 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose, and 10 mM HEPES titrated to pH 7.4 with NaOH (310 mOsm). The patch pipette solution contained 120 mM KCl, 1 mM $MgCl_2$, 3 mM Mg-ATP, 10 mM HEPES, and 10 mM EGTA titrated to pH 7.2 with KOH and adjusted at 290 mOsm with saccharose. All materials were purchased from Sigma-Aldrich. Axopatch 200B, Digidata 1322A, and pCLAMP 10 (Molecular Devices, Sunnyvale, CA) were used for data amplification, acquisition, and analysis. Signals were digitized at 10 kHz and filtered at 2 kHz. Microelectrodes with resistances of 4–7 MΩ were pulled from borosilicate glass capillaries (Harvard Apparatus, Holliston, MA). The electrical signal obtained from the contracting cardiomyocyte was sampled at 10,000 Hz and downsampled to 200 Hz. The recordings were analyzed for the detection of peaks of the recorded signal from which interbeat intervals (IBIs) were calculated by using the dedicated MATLAB software.

Data analysis

We used 3 different means to analyze BRV/HRV (see the Online Supplement): (1) the detrended fluctuation analysis (DFA), which quantifies the intrinsic fractal-like behavior of nonstationary time series (Online Supplemental Figure 1A)[6,11]; (2) Poincaré plot, in which each IBI (IBI_{n+1}) is plotted against its predecessor (IBI_n), reflecting the dynamics of the system[12]; and (3) power-law analysis (see the Online Supplement), which determines the scaling relationship of the process.[13]

Statistical analysis

See the Online Supplement.

Results

IBI variability occurs at the 3 levels

Figure 1 demonstrates the ECG of a 39-year-old healthy female volunteer (#201201) from whom iPSCs were generated, the EB electrogram, and single cardiomyocyte action potentials. Pronounced BRV/HRV at all 3 levels is illustrated by the IBI vs time plot and by IBI histograms. Similar findings from the second volunteer (#201202) are presented in Online Supplemental Figure 2. The fact that the cells within the entire EB network beat in synchrony and are