Action Potential Morphology of Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes Does Not Predict Cardiac Chamber Specificity and Is Dependent on Cell Density

Supporting Material

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Supplementary information

Methods

Cardiomyocyte preparation

iPSC-CMs (iCell Cardiomyocytes – Cellular Dynamics International, Madison, WI, USA) were plated as per manufacturer's protocol onto 14 mm glass-bottomed dishes (MatTek, USA). iPSC-CMs were plated at two different seeding densities: 60,000 cells/well (confluent monolayer) and 15,000 cells/well (sparsely-seeded). Cells were incubated at 37°C in 5% CO_2 before use 14 days from seeding. Subsequent experiments were performed to assess the effect of gap junction communication in confluently-seeded preparations on action potential morphology. iPSC-CM action potentials were assessed, as below, before and after the application of 20 μM carbenoxolone (Sigma Aldrich) (1).

Imaging

Action potential morphology was assessed using the voltage-sensitive fluorescent dye di-8-ANEPPS (Invitrogen). iPSC-CMs were loaded with 5 μM di-8-ANEPPS in 1 ml DMEM at 37°C for 20 minutes. 4 μM blebbistatin was added 5 minutes before recording to inhibit excitation-contraction coupling and prevent signal distortion due to motion artefact. Di-8-ANEPPS was excited using a 535-nm LED and the emitted fluorescence collected through a 590-nm long-pass filter. The cells were electrically field stimulated at 1 Hz with a 20±10V stimulus of 5 ms duration and five-second recordings were captured using a NeuroCMOS camera (Redshirt) at 1 kHz using a ×40 oil-immersion objective. Dishes were kept at 37°C in normal Tyrode's solution (in mM: 140 NaCl, 4.5 KCl, 10 Glucose, 10 HEPES, 1 $MgSO_4$, 1.8 $CaCl_2$ – pH 7.4) throughout recording. Six dishes were studied in the confluent group, with 2-7 recordings taken per dish. Two dishes were studied for the sparse group, with 1-4 recordings taken per dish. Four dishes were studied for the carbenoxolone experiments, with 5-6 recordings taken per dish.

Image processing

Recordings were captured at 128 × 128 pixels. Individual cardiomyocytes were selected post-hoc in Optiq (Dr Francis Burton, University of Glasgow, Glasgow, UK) using a rectangular selection tool. In confluent monolayer each cardiomyocyte selection had sizes ranging from approximately 10 × 10 – 17 × 17 pixels, corresponding to approximately 31.25 × 31.25 – 53.125 × 53.125 μm. In sparsely seeded dishes, each cardiomyocyte selected had a size ranging from approximately 25 × 25 – 56 × 56 pixels, corresponding to 78.125 × 78.125 – 175 × 175 μm. Sparse cells were in partial contact with each other. AP analysis software, Optiq, was used for selection of cardiomyocytes and for curve fitting and automated measurement of AP parameters: amplitude, time to peak, APD_{90}, and APD_{50} (Fig. 1, A, B and C). Repolarization was measured with respect to the maximum diastolic potential of each

AP. Parameter measurements from three consecutive APs per cardiomyocyte were recorded and averaged for analysis. iPSC-CMs that could not be paced at 1 Hz were not assessed.

Statistical analysis

Statistical analysis of APD_{90} and repolarization was performed using Prism6 (GraphPad Software Inc., San Diego, CA, USA) and parametric tests of variance (F-test) were performed using MedCalc 14.8.1 (MedCalc Software bvba, Ostend, Belgium). The non-parametric two-tailed unpaired Kolmogorov-Smirnov (K-S) test was used to determine statistical differences in distribution. Student's *t*-test with Welch's correction was used to determine differences in mean APD in the carbenoxolone experiments. $P<0.05$ was considered statistically significant. Data are expressed as mean (SD). n signifies the number of cardiomyocytes analyzed. No assumptions were made with respect to the distribution of the populations. Parametric and non-parametric tests were performed due to the relatively low number of sparse cells producing equivocal distributions.

Limitations

(1) The use of blebbistatin for inhibition of mechanical function. However, there is conflicting evidence as to whether or not blebbistatin affects APD (2, 3). Both of these reports used Langendorff-perfused whole hearts to optically measure APD; whether APD prolongation is due to myocytes being mechanically unloaded, or is a direct effect of blebbistatin on the cardiomyocyte, however, cannot be established.

(2) The use of a single source of iPSC-CMs; future studies will test iPSC-CMs from different manufacturers using different culture mediums and culture times.

References for supporting material:

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(2) Brack, K.E., R. Narang, J. Winter, and G.A. Ng. (2013). The mechanical uncoupler blebbistatin is associated with significant electrophysiological effects in the isolated rabbit heart. Exp. Physiol. 98: 1009–27

(3) Fedorov, V. V, Lozinsky, I. T., Sosunov, E. a, Anyukhovsky, E. P., Rosen, M. R., Balke, C. W., & Efimov, I. R. (2007). Application of blebbistatin as an excitation contraction uncoupler for electrophysiologic study of rat and rabbit hearts. Heart Rhythm : The Official Journal of the Heart Rhythm Society, 4(5), 619–26