Supplementary Information for

Mechano-electric coupling and arrhythmogenesis in cardiomyocytes contracting under mechanical afterload in a 3D viscoelastic hydrogel

Bence Hegyi[1], Rafael Shimkunas[1,2], Zhong Jian[1], Leighton T. Izu[1], Donald M. Bers[1], Ye Chen-Izu[1,2,3,*]

[1]Department of Pharmacology, University of California, Davis, CA 95616, USA
[2]Department of Biomedical Engineering, University of California, Davis, CA 95616, USA
[3]Department of Internal Medicine/Cardiology, University of California, Davis, CA95616, USA

*Corresponding Author: Ye Chen-Izu, PhD
Email: ychenizu@ucdavis.edu

This PDF file includes:

Supplementary text

Figures S1 to S4

Supplementary Information Text

Extended Methods

Animal model and cell isolation

Twenty young adult (3–to 4-month-old, male, 2.5–3 kg) New Zealand White rabbits (Charles River Laboratories, Wilmington, MA, USA) were used for experiments. Rabbits were fed by standard pellet food and water ad libitum, both were checked and changed every day. Fifteen min before terminal surgery rabbits were injected with heparin (400 U/kg, I.V.), then anesthetized with isoflurane (3-3.5%) inhalation. After achieving deep anesthesia, hearts were quickly excised, and placed in a cold Tyrode's solution. Then the hearts were mounted on a Langendorff apparatus and retrogradely perfused for 5 min with oxygenated Tyrode's solution to remove blood from the coronary vasculature. Then a Ca^{2+}-free Tyrode's solution was perfused for 3 min to stop the contraction of the heart. Next, this solution supplemented with 1 g/L type II collagenase (Worthington, Lakewood, NJ, USA), 0.05 g/L protease type XIV (Sigma-Aldrich, St. Louis, MO, USA), and 50 µmol/L Ca^{2+} was perfused for 25-30 min to enzymatically dissociate cells. After perfusion, both atria and the right ventricle were removed, the left ventricle was minced, and ventricular cells were then harvested and stored in a modified Tyrode's solution with the following composition (in mmol/L): NaCl 124, $NaHCO_3$ 25, KCl 4, $CaCl_2$ 1.2, $MgCl_2$ 1, HEPES 10, glucose 10, pH=7.4 (adjusted with NaOH).

Electrophysiology

Freshly isolated rabbit ventricular cardiomyocytes were first suspended in PVA (10 wt.%), then placed in a Plexiglas chamber and perfused with a bicarbonate-containing modified Tyrode's solution having the following composition (in mmol/L): NaCl 120, $NaHCO_3$ 25, KCl 4, $CaCl_2$ 1, $MgCl_2$ 1, HEPES 10, pyruvic acid 5, taurine 10; pH=7.4 (adjusted with NaOH). Electrodes were fabricated from borosilicate glass (World Precision Instruments Inc., Sarasota, FL, USA) having tip resistances of 2.5–3 MΩ when filled with internal solution containing (in mmol/L): K-Aspartate 110, KCl 25, NaCl 5, Mg-ATP 3, HEPES 10, cAMP 0.002, phosphocreatine dipotassium salt 10, and EGTA 0.01; pH=7.2 (adjusted with KOH). This ionic composition preserves cardiomyocyte contractions and intracellular Ca^{2+} transients. The osmolality of all applied solutions was carefully adjusted to 298±3 mOsm using a vapor pressure osmometer (Vapro 5520, Wescor Inc., Logan, UT, USA). Action potentials (APs) and ionic currents were recorded using the whole-cell configuration of the patch-clamp technique. The cardiomyocytes were continuously stimulated in current-clamp experiments with supra-threshold depolarizing pulses (2-ms duration) delivered via the patch pipette at 0.5 Hz frequency. Formation of 3D viscoelastic hydrogel around the contracting cardiomyocyte was then achieved applying 4B-PEG crosslinker. Axopatch 200B amplifier (Axon Instruments Inc., Union City, CA, USA) was used for patch-clamp experiments and the signals were digitized at 50 kHz using Digidata 1440A A/D converter (Molecular Devices, San Jose, CA, USA) under pClamp 10.4 software control (Molecular Devices). The series resistance was typically 4-5 MΩ before compensation (usually 90%). Experiments were discarded when the series resistance was high or increased by >20% during the recordings. Reported voltages are already corrected for liquid junction potentials. All experiments were conducted at room temperature (22±1°C).

L-type Ca^{2+} current ($I_{Ca,L}$) was measured using a depolarizing voltage step to +5 mV from a holding potential of −40 mV to inactivate Na^+ channels. Tyrode's solution was supplemented with 3 mmol/L 4-aminopyridine and 1 µmol/L E 4031 to inhibit transient outward K^+ current (I_{to}) and rapid delayed rectifier K^+ current (I_{Kr}), respectively. At the end of the experiment, $I_{Ca,L}$ was inhibited using 10 µmol/L nifedipine.

Inward rectifier K^+ current (I_{K1}) was measured using a hyperpolarizing voltage step to −140 mV. Steady-state I_{K1} density was analyzed in the end of voltage step. At the end of the experiment, I_{K1} was inhibited using 300 µmol/L Ba^{2+}.

Ionic currents were normalized to cell capacitance, determined in each cell using short (10 ms) hyperpolarizing pulses from −10 mV to −20 mV. Capacitance of ventricular cardiomyocytes was 148.6±0.9 pF (n = 96 cells from 20 animals).

Chemicals and reagents were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) if not specified otherwise. L-NPA, L-NIO and E-4031 were from Tocris Bioscience (Bristol, UK). GsMTx-4 was from Alomone Labs (Jerusalem, Israel).

Cytosolic and SR Ca^{2+} concentration measurements

Intracellular (cytosolic) Ca^{2+} concentration, $[Ca^{2+}]_i$, was measured using the Fura-2 ratiometric method. Freshly isolated cardiomyocytes were loaded in Tyrode's solution with the membrane permeant form of Fura-2 (Fura-2AM, 2.5 µmol/L) in the presence of Pluronic F127 (0.75 µmol/L in 20% DMSO) for 30 min at room temperature, then washed for 15 min, and used for experiments within 4 hours. An IonOptix system (IonOptix, Inc., Westwood, MA, USA) with a HyperSwitch was mounted on an Olympus X71 inverted microscope with a water-immersion fluorescence objective (UPlanSApo 40X, NA 1.15) positioned under the imaging chamber and corrected for glass coverslip (No. 1) thickness. The excitation light was generated using a xenon arc lamp. The galvanometer-based HyperSwitch delivered dual-excitation beams at 340 and 380 nm using a 340/370d/380 filter cube, switching between the two wavelengths at 0.5 kHz frequency. The emission fluorescence passed through a D510/40m bandpass filter and captured in a photo multiplier tube (PMT). The whole cell fluorescence signal was acquired by closing the camera aperture around a single cell and measuring the emitted fluorescence from both excitation wavelengths. Background fluorescence for both the gel and the Tyrode solution was measured from an area adjacent to the cell for each recording. The Fura-2 fluorescence ratio was then calculated from the fluorescence emissions from cells at 340 and 380 nm excitation wavelengths after background subtraction. Cells were paced at 0.5 Hz frequency at room temperature.

Intra-sarcoplasmic reticulum (SR) Ca^{2+} concentration, $[Ca^{2+}]_{SR}$, was measured using the low-affinity Ca^{2+} indicator Fluo-5N. Freshly isolated rabbit ventricular myocytes were loaded with Fluo-5N in Tyrode's solution with 15 µmol/L Fluo-5N-AM and 2.5 µmol/L Pluronic F127 (20% in DMSO), incubated for 2 hours at 37°C, washed and then incubated for 1.5 hours with fresh Tyrode's solution at 37°C. The excitation light passed through a D480/30X bandpass filter, and the emitted light passed through a D535/40M bandpass filter before captured in a PMT. Background-corrected Fluo-5N fluorescence was obtained in the same cell in Tyrode's solution and in-gel. Cells were paced at 0.5 Hz frequency at room temperature.

Cardiomyocyte contraction measurements

Sarcomere shortening was measured simultaneously with Ca^{2+} measurements using the IonOptix sarcomere detection and fast Fourier transform (FFT) method in paced cardiomyocytes. To record sarcomere movement during cardiomyocyte contraction an IonOptix system (IonOptix, Inc.) with a high-speed camera (MyoCam-S, 1000 frames/s) was used. The sarcomere length (SL) was calculated using a fast Fourier transform algorithm. The fractional shortening (FS) was then calculated as the percentage of change in SL during contraction, FS(%) = (diastolic SL–systolic SL)/diastolic SLx100.

Statistical Analysis

Data are expressed as the mean ± SEM. Statistical significance of differences was evaluated using two-tailed Student's *t*-test (paired or unpaired) to compare two groups, and analysis of variance (ANOVA) to compare multiple groups with Dunnett's or Tukey's *post-hoc* tests as indicated. Normality of the data was assessed by Shapiro-Wilk test and the equality of group variance was tested using Brown-Forsythe test. Representative traces reflected the average level of each experiment. The number of cells and animals in each experimental group is reported in the figure legends. Group sizes were determined by an *a priori* power analysis for a two-tailed Student's *t*-test with an α of 0.05 and power of 0.8, in order to detect a 10% difference signal at the endpoint. Animals were grouped with no blinding but randomized in experiments. Fully blinded analysis was not performed because the same person carried out the experiments and analysis. Analysis, graphing, and statistical evaluation of data were carried out using Excel 2016 (Microsoft, Redmond, WA, USA), Prism 8 (GraphPad Software, San Diego, CA, USA) and Origin 2016 software (OriginLab Co., Northampton, MA, USA). $P<0.05$ was considered statistically significant.

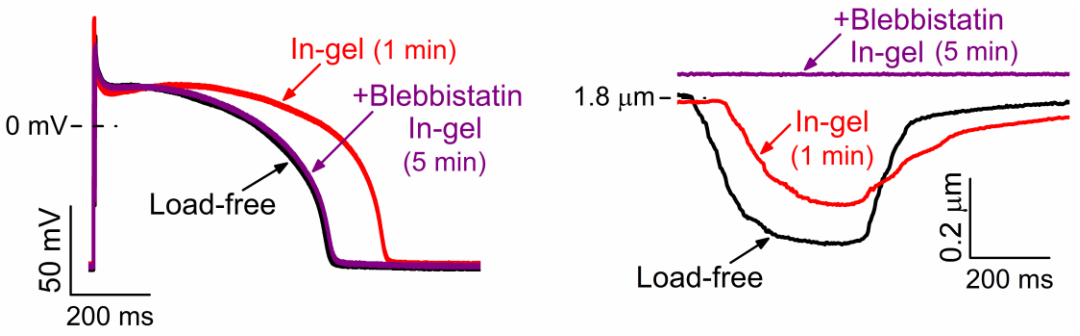

Fig. S1. Mechanical afterload induced changes in action potential are fully reverted by blebbistatin. Treatment with the myosin II ATPase inhibitor blebbistatin (10 μmol/L, 5 min) stopped cardiomyocyte contraction (thus removing the mechanical load in-gel) and reverted the changes in action potential waveform. The cardiomyocyte was continuously paced at 0.5 Hz frequency throughout the experiment.

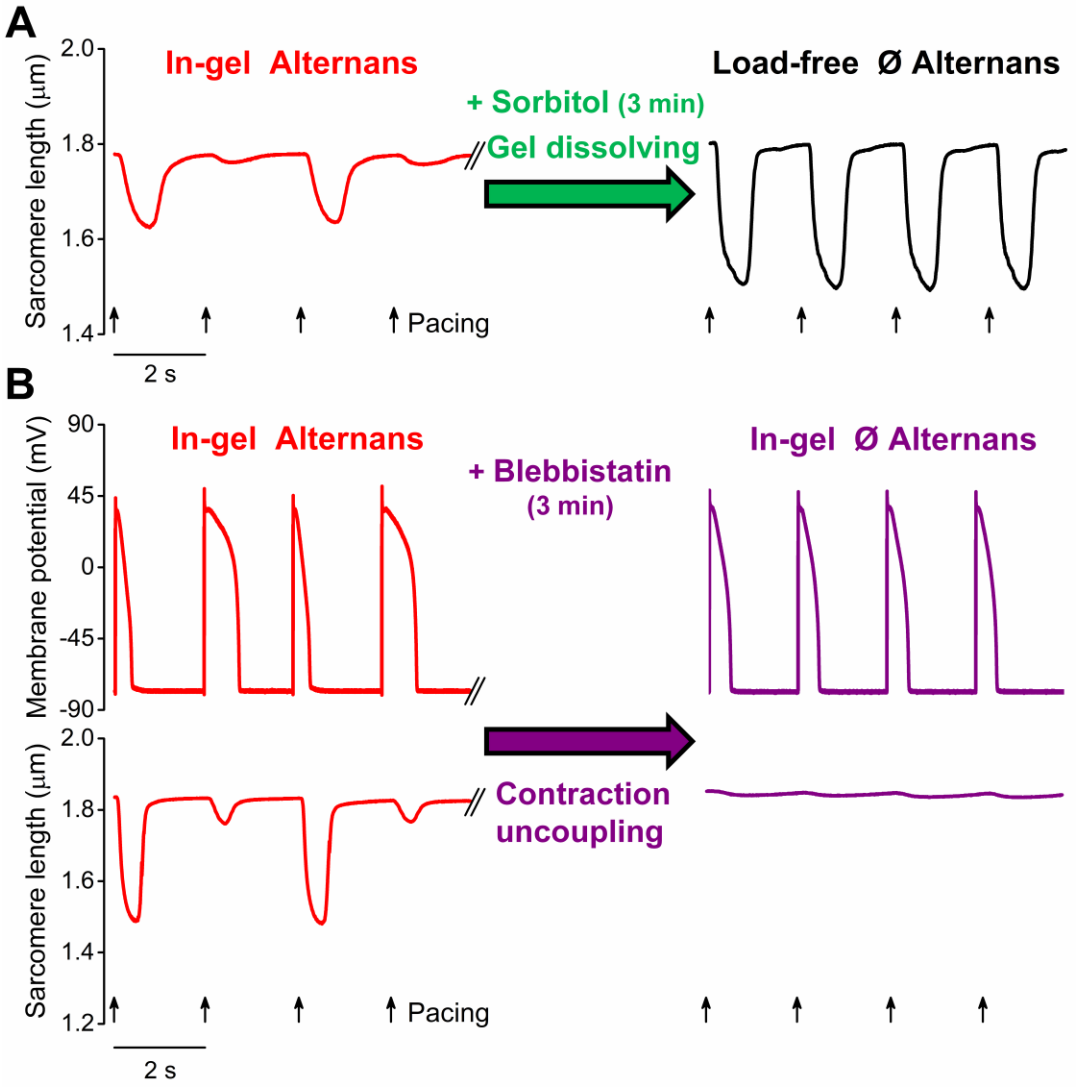

Fig. S2. Cardiac alternans is abolished by removing the mechanical load. *(A)* Perfusion with sorbitol (1 wt.%, 3 min)-containing Tyrode's solution to dissolve the crosslinked gel (i.e., removing the mechanical afterload during systolic contraction) abolished the contraction alternans. *(B)* Treatment with the contraction uncoupler blebbistatin (10 μmol/L, 3 min) abolished the alternans in action potential duration. Cells were paced at 0.5 Hz frequency.

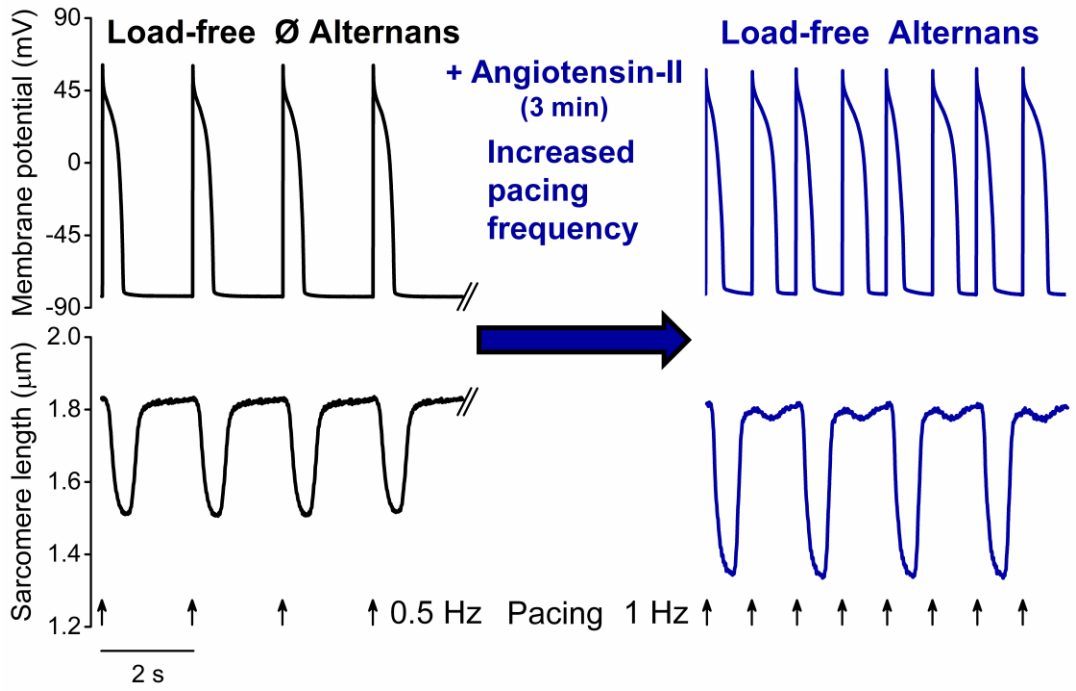

Fig. S3. Cardiac alternans induced by angiotensin II. Treatment with angiotensin-II (1 µmol/L, 3 min) and increasing the pacing rate (from 0.5 Hz to 1 Hz) induced discordant alternans in action potential duration (short-long-short-long) and contraction (large-small-large-small) under load-free conditions.

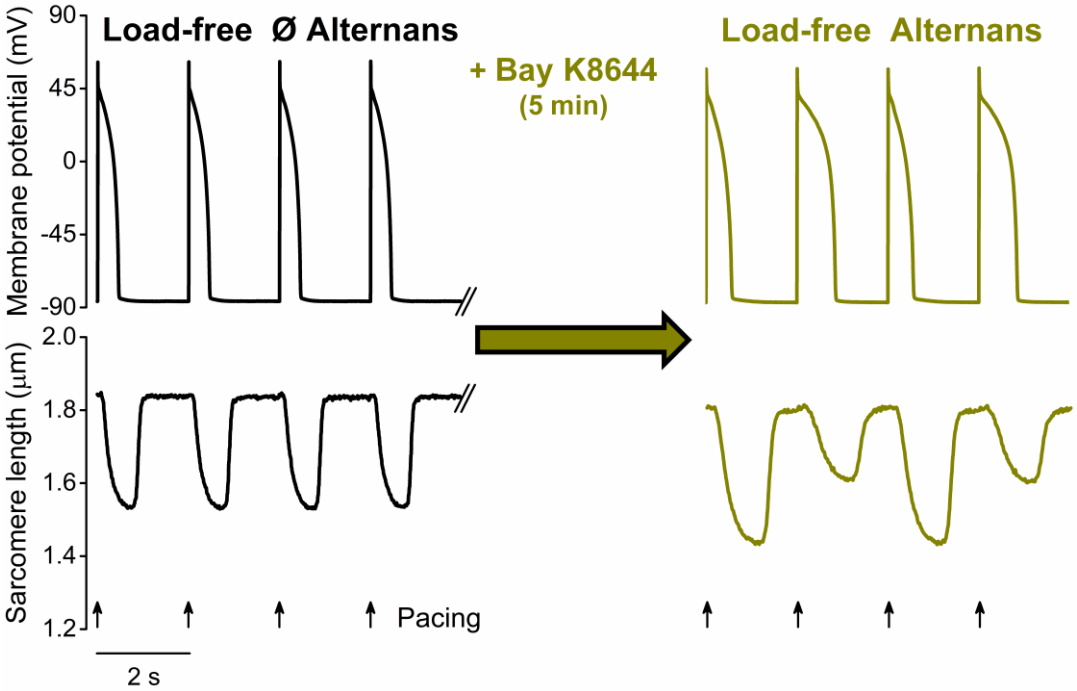

Figure S4. Cardiac alternans induced by L-type Ca^{2+} current augmentation. Treatment with Bay K8644 (100 nmol/L, 5 min) induced discordant alternans in action potential duration (short-long-short-long) and contraction (large-small-large-small) under load-free conditions. Cells were paced at 0.5 Hz frequency.