Supporting Information

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SI Materials and Methods

All animal surgical procedures and perioperative management were carried out in accordance with the United Kingdom Home Office Guide on the Operation of the Animals (Scientific Procedures) Act 1986, which conforms to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (publication no. 85–23, revised 1996).

Rat Heart Failure Model. Adult male Sprague-Dawley rats (250–300 g) ($n = 6$) underwent proximal coronary ligation to induce chronic myocardial infarction. Sham ligation was used as control ($n = 6$). Sixteen weeks later, in vivo PV analysis was performed using the 2-F Millar microconductance catheter (SPR838; Millar Instruments) via an apical approach under isoflurane (1.5%) anesthesia. Steady-state data (LVEDV, LVEDP, and LVEF) were recorded after 15 minutes' stabilization. Load-independent measurements were recorded during transient intrathoracic inferior vena cava occlusion (left ventricular ESPVR, left ventricular EDPVR, left ventricular Emax, and PRSW). Parallel conductance was measured after i.v. 50-μL hypertonic saline (15%) injection. Data were recorded using CHART 5.5 software (AD Instruments) and analyzed offline using PVAN 3.6 software (Millar Instruments). At completion of the procedure, and before killing, blood (2-mL) samples were taken for cuvette calibration and BNP analysis using rat BNP-32 ELISA kit (S-1251; Peninsula Laboratories). Hearts were explanted, weighed, and prepared for cell isolation.

Human Cardiomyocytes. Human myocardium was obtained from explanted hearts of patients with IHD or DCM, or septal myectomy specimens from patients with HOCM. Nonfailing cardiomyocytes were sourced from unused donor hearts or biopsies from ventricles of patients undergoing coronary artery bypass surgery with normal LVEF (>60%). For the detubulation/SICM data, 3 hearts were used from each of the donor, IHD, DCM, or HOCM groups: numbers given in results and figure legends are total numbers of myocytes tested.

Cell Isolation Protocol. Myocytes were isolated from male Sprague-Dawley rats by the Langendorff perfusion method, as previously described (1, 2). Human myocytes were isolated by chopping of tissue and incubation in low-calcium and collagenase/protease-containing solutions (3).

Scanning Ion Conductance Microscopy. SICM is a scanning probe microscopy technique that uses a glass nanopipette as a sensitive probe that detects ion current and uses the current as an interaction signal to control the vertical (z axis) position of the cell relative to the pipette tip (4, 5). Contractile characteristics of single myocytes were measured using the IonOptix system, and cells from the same preparation were imaged using the SICM, which produces a 3-dimensional topographic representation of the cell surface.

T-Tubule Labeling T-tubule density was measured after sarcolemmal labeling with Di-8-ANEPPS. Ventricular myocytes were incubated with 10 μM Di-8-ANNEPS (Molecular Probes) for 15 min and then washed for 30 min before being observed under the confocal microscope (6). After Di-8-ANEPPS labeling the density of T-tubules was quantified by the ratio of T-tubule fluorescence (T-tubule membrane) to total plasma membrane fluorescence (total membrane) in the same confocal slice, with excitation at 488 nm and emission detected at 520 nm (7).

Z-Groove Ratio Calculation. To quantify the data obtained during scanning, we introduced an index of the completeness of the Z-grooves on the surface of cardiomyocytes (Z-groove ratio). To calculate Z-groove ratio, we divided the length of Z-grooves seen on a single image by the total estimated Z-groove length, as if they all were present on the surface, as described previously (1).

Measurement of Cardiomyocyte Contraction. Myocytes in suspension were placed into a Perspex bath with a glass floor on the stage of an inverted microscope and superfused with Krebs-Henseleit solution (1 to 2 mM Ca^{2+}) equilibrated with 95% O_2/5% CO_2 at 37 °C (rat) or 32 °C (human). Cells were electrically stimulated at 0.5 Hz (rat) and 0.2 Hz (human), and cell shortening with each beat followed using the IonOptix system, scanning at 120 Hz. Contraction amplitude (percent shortening), TTP, and R50 were analyzed offline (2, 3).

Contractile measurements quoted for nonfailing heart were taken from previous cohorts (8), whereas those from HOCM, IHD, or DCM were from a subset of preparations used for SICM.

Calcium Sparks and SR Release Events. The Ca^{2+}-sensitive fluorescent dye Fluo-4 was used to monitor localized changes in cytoplasmic [Ca^{2+}]. Aliquots of cells were incubated with Fluo-4 AM (10 μM) for 15–20 min. Supernatant was then discarded and substituted with DMEM (Gibco BRL, Life Technologies). The cells were not used in experiments for at least 30 min to allow de-esterification of the intracellular indicator. The experimental chamber was mounted on the stage of a Nikon TE300 microscope with Bio-Rad Radiance 2000 confocal attachment and myocytes observed through a Nikon Plan-Fluor ×40 oil-immersion lens (N.A. = 1.3). Fluo-4 was excited using the 488-nm line of an argon laser and the emitted fluorescence collected through a 520-nm filter.

Ten thousand line scans were collected (512 pixels wide scanned at 1.3-ms intervals) for analysis with both ImageJ (Scioncorp) and LaserPix software (Bio-Rad). Detection criteria for Ca^{2+} sparks were set at 3.7 × SD, and automated counting of sparks was performed using the Sparkmaster plug-in for ImageJ (10).

To investigate the extent of synchronous release, Ca^{2+} transients were recorded during steady-state stimulation at 0.5 or 1 Hz along a line placed near the center of the cell and parallel to its long axis. The temporal resolution was 1.33 ms per line, and pixel size (x,y) was 0.2–0.4 μm. A method similar to that of Heinzel et al. (9) was used. Poor release was defined as pixel values less than half the maximum of the normalized overall peak Ca fluorescence (F_{50} value). This value was regarded as the threshold to discriminate local Ca release. Lines 20 ms from the start of the transient were examined, and sections of these lines where the fluorescence did not reach F_{50} were counted and defined as the number of delayed sites.

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