SUPPLEMENTAL MATERIAL

Methods

This study protocol was approved by the Institutional Animal Care and Use Committee of Indiana University School of Medicine and the Methodist Research Institute, and conforms to the Guide for the Care and Use of Laboratory Animals.[1] New Zealand white female rabbits (N=27) were used. We attempted to induce HF in 10 rabbits by rapid pacing. Among them, 7 completed the pacing protocol and developed HF. The remaining 3 rabbits died 1.7 [95% CI, 0.2 to 3.1] days after the onset of tachycardia pacing at 250 bpm. We studied 7 normal rabbit hearts as controls. The other 10 hearts were used for western blot analyses (5 with pacing-induced HF and 5 normal controls).

Pacing-induced Heart Failure

Rapid ventricular pacing was used to induce HF.[2] Surgery was performed with isoflurane general anesthesia. After left lateral thoracotomy, an epicardial pacing lead was sutured at the lateral wall of left ventricle and connected to a modified single chamber ventricular pacemaker (Kappa or Enpulse pacemaker, Medtronic, Inc., Minneapolis, MN, USA) for tachycardia pacing. After 1 week of convalescence, the ventricles were paced at 250 bpm for 3 days, 300 bpm for 3 days, and 350 bpm for 3-5 weeks to induce HF. Left ventricular dimension and systolic function were assessed by echocardiography before surgery and after 3-5 weeks of ventricular pacing.

Optical Mapping

The rabbits were intravenously injected with 1,000 units of heparin and anesthetized with sodium pentobarbital (35 mg/kg). After a median sternotomy, the hearts were rapidly excised and Langendorff perfused at 25 to 35 mL/min with oxygenated Tyrode's solution (in mmol/L: NaCl 125, KCl 4.5, $NaHCO_3$ 24, NaH_2PO_4 1.8, $CaCl_2$ 1.8, $MgCl_2$ 0.5, and glucose 5.5) with a pH of 7.40. The hearts were stained with Rhod-2 AM (1.48 μmol/L) for Ca_i and RH237 (10 μmol/L) for Vm mapping. The double-stained hearts were illuminated with a laser at 532 nm

wavelength. The fluorescence was filtered and recorded simultaneously with dual CMOS cameras (Brain Vision, Tokyo, Japan) at 2ms/frame and 100x100 pixels with a spatial resolution of 0.35x0.35 mm^2 per pixel. The fluorescence obtained through a common lens was separated with a dichroic mirror (650 nm cutoff wavelength), and directed to the respective camera with additional filtering (715 nm long pass for Vm and 580±20 nm for Ca_i). Optical signals were processed with both spatial (3X3 pixels Gaussian filter) and temporal (3 frames moving average) filtering. Phase mapping was performed to evaluate the location and evolution of phase singularities (PSs). Blebbistatin (10-20 μmol/L, Tocris, Ellisville, MO) was used to inhibit motion artifact during optical mapping.

Experiment Protocol

A pair of hook bipolar electrodes was inserted into the posterior wall of right ventricle for pacing. A pseudo-ECG was obtained with widely spaced bipolar electrodes to determine ventricular rhythm. S_1 dynamic pacing protocol (2X diastolic threshold) was used to determine the APDR at baseline and after 30-min apamin infusion (100 nM). The ventricles were initially paced at a constant PCL of 350 ms. The PCLs were progressively shortened (350, 300, 280, 260, 240, 220, 200, 190, 180, 170, 160, 150 ms) until VF was induced or the loss of 1:1 capture of the ventricles. Optical recording was performed after 30 beats of stable pacing at each PCL. If VF was not induced by the dynamic pacing protocol, 2-3 attempts of burst pacing (PCL 50-100 ms, pacing duration 5-10 s) was used to test whether or not VF was inducible. The same protocol was used to test VF inducibility before and after apamin infusion in both normal and HF ventricles. Optical recordings were then performed during VF. We allowed VF to continue for at least 180 s before defibrillation.

Western Blotting

Myocardial blocks from left ventricles (5 HF and 5 normal controls) were excised and chopped immediately after harvesting. 100 mg tissues were homogenized by POLY TRON in 1 ml RIPA buffer with protease inhibitor (50 mM Tris pH 8.4, 150 mM NaCl, 1% NP40, 0.5%

sodium deoxycholate 1 mM PMSF, 2 μg/ml leupeptin, 1 μg/ml pepstatin A, and 5 μg/ml aprotinin). Homogenates were incubated on ice for 30 min and then centrifuged at 14,000 rpm for 15min. 20 μg of supernatants were subjected to electrophoresis using Bio-Rad mini gel system. The separated proteins were transferred to PVDF (Millipore). The membrane was bathed in TBS with 5% milk for one hour, and probed with either anti-KCNN2 antibody (for detecting SK2 channel, Abcam, ab83733, 1:2500) or anti-GAPDH antibody (PIERCE, MA1-22670, 1:2500) overnight. After the interaction with primary antibody, the membrane was incubated with HRP-conjugated anti-rabbit or anti-mouse secondary antibodies (sigma, 1:5000) for 30 min. Finally, Luminata Crescendo HRP substrate (Millipore, WBLUR100) was added onto the membrane according to manufacturer's instruction.

Data Analysis

Construction of APDR Curves

Optical APD_{80} was measured at 80% repolarization. The APD_{80} was measured by computerized methods using all available pixels on the ventricles, excluding the atria and the pixels at the edge of the ventricles. APDR curve was constructed by plotting APD_{80} against the preceding diastolic interval (DI), defined by the interval between 80% repolarization and the onset of the next action potential. APD alternans was defined as the difference in APD_{80} of 2 consecutive beats of ≥4 ms during dynamic pacing. Both long and short APDs observed during alternans are included in plotting APDR curve. The slopes of APDR were calculated by first-order exponential fitting with ORIGIN software (Microcal). Two-dimensional (2D) APD_{80} maps were constructed to study the spatial distribution of APDs on the epicardial surfaces of the hearts. We also analyzed the correlation between baseline APD_{80} and delta APD (apamin-treated APD_{80} minus baseline APD_{80}) at PCL of 350, 300, and 260 ms.

Fast Fourier Transforms (FFTs) Analysis and Epicardial Wavebreaks during VF

FFTs of pseudo-ECGs (4 s in duration) were used to determine the dominant frequency (DF) of VF at baseline and after apamin infusion.[3, 4] For each optical recording, optical data were

acquired continuously for 4.096 s (2048 frames). A PS shown on the phase maps was defined as a site with an ambiguous phase surrounded by pixels exhibiting a continuous phase progression from –π to +π. Previous studies suggest that PSs are a robust alternate representation of wavebreaks,[5] which serve as the source of VF. To quantify wavebreaks during VF, the numbers of PSs in the phase map were counted manually every 10 frames for 1,000 frames in each episode of VF.

Statistical Analysis

Data are presented as mean and 95% confidence interval (CI). Paired and unpaired Student's t-tests were used to compare the data within and between groups. Categorical parameters between groups were compared by Fisher's exact test. A two-sided p-value of ≤ 0.05 was considered significant.

Results

SK2 Protein

Western blotting was performed in a separate group of 5 normal control and 5 failing hearts which were not used for optical mapping. Immunoblot in Figure A shows antibody weakly identified expression of SK2 channels in these samples. Figure B shows the SK2/GAPDH ratio of all hearts studied. The difference between normal and failing ventricles was not statistically significant (for at least 3 measurements, p=0.078).

References

1. National Research Council Committee. *Guide for the care and use of laboratory animals*. Washington (DC): National Academies Press (US); 2011.
2. Chua SK, Chang PC, Maruyama M, Turker I, Shinohara T, Shen MJ, Chen Z, Shen C, Rubart-von der Lohe M, Lopshire JC, Ogawa M, Weiss JN, Lin SF, Ai T, Chen PS.

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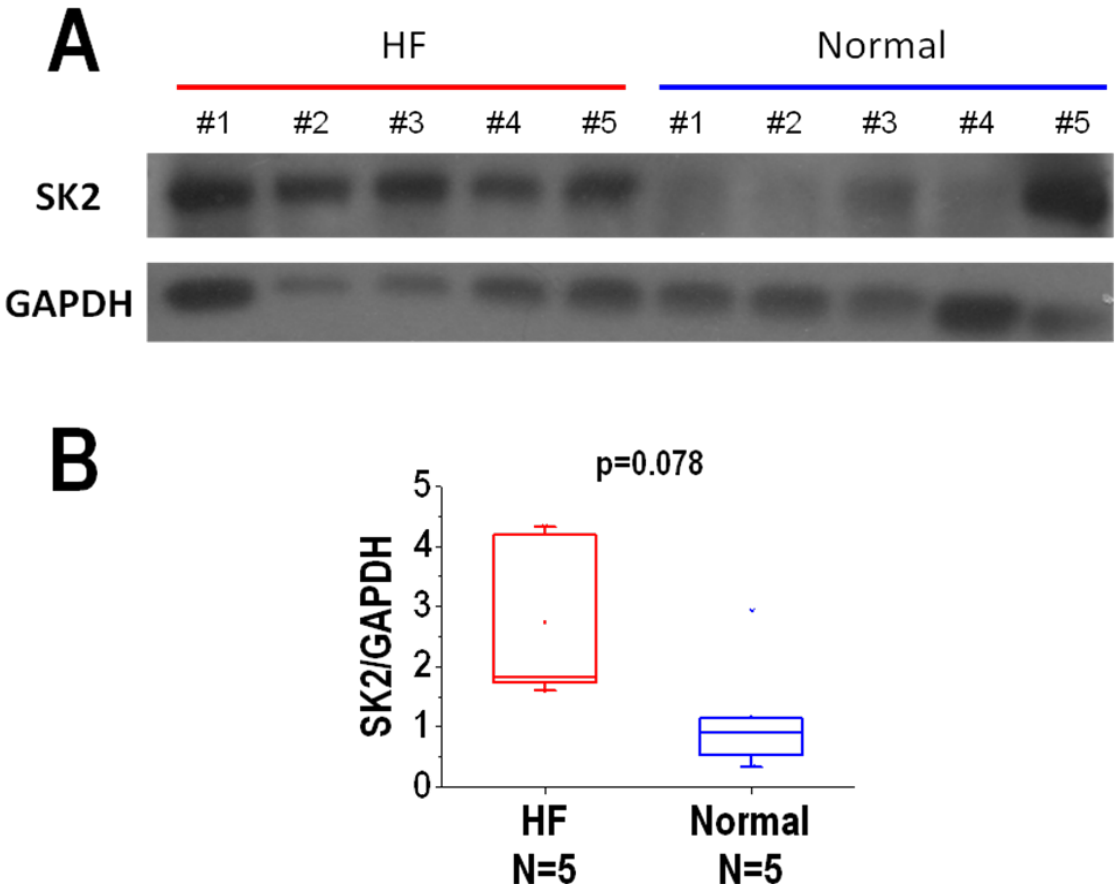
3. Mandapati R, Asano Y, Baxter WT, Gray R, Davidenko J, Jalife J. Quantification of effects of global ischemia on dynamics of ventricular fibrillation in isolated rabbit heart. *Circulation*. 1998;98:1688-1696.
4. Wu TJ, Lin SF, Weiss JN, Ting CT, Chen PS. Two types of ventricular fibrillation in isolated rabbit hearts: Importance of excitability and action potential duration restitution. *Circulation*. 2002;106:1859-1866.
5. Liu YB, Peter A, Lamp ST, Weiss JN, Chen PS, Lin SF. Spatiotemporal correlation between phase singularities and wavebreaks during ventricular fibrillation. *J Cardiovasc Electrophysiol.* 2003;14:1103-1109.

Online Supplement Figure 1. SK2 protein analysis. A. An example of immunoblot shows that the anti-SK2 antibody weakly identified expression of SK2 channels in these samples. B. Plot shows a compilation of the SK2/GAPDH ratio of all ventricles studied (at least 3 measurements). Note that there was statistically insignificant increase of the SK2/GAPDH ratio in HF ventricles as compared with normal ventricles. SK2, small conductance Ca-activated K channel subtype 2.


A
HF
Normal
#1
#2
#3
#4
#5
#1
#2
#3
#4
#5
SK2
GAPDH
B
p=0.078
SK2/GAPDH
0
1
2
3
4
5
HF
N=5
Normal
N=5