MATERIALS AND METHODS

Canine Model of Sustained Ventricular Tachyarrhythmia

The model has been previously described in considerable detail.[1-3] Left circumflex coronary artery occlusion (2min) started the last minute of submaximal exercise was used to test animals for ventricular fibrillation (VF) susceptibility. Large defibrillation (Adult Statz-pads; Zoll Medical, Burlington, MA) electrodes were placed across the animal's chest so that electrical defibrillation could be achieved with a minimal delay, but only after the animal was unconscious (10-20 s after VF onset).

Myocardial infarction size was not measured in the present study, inasmuch as the hearts were removed for the *in vitro* studies. However, in previous studies using this canine model of sudden death, the ligation of left anterior coronary artery produced modest myocardial infarction in the dogs shown to be susceptible to VF (17.7 ± 0.9 % of left ventricular mass, n = 93).[3]

Optical Action Potential (AP) Mapping in the Canine Wedge Preparation

Details of the experimental procedure for optical mapping in the canine wedge preparation are provided elsewhere.[4] Physiological stability of the preparations was insured by monitoring the volume-conducted electrocardiogram, coronary perfusion pressure (50-60 mmHg), coronary flow, and perfusion temperature (35.5 ±0.5°C) continuously throughout each experiment.

Activation times, repolarization times, and AP duration (APD) were measured directly from all optical APs using previously validated algorithms.[4] APD alternans magnitude was defined as the difference in APD between two consecutive beats [5] during stable alternans. Stimulation rate causing APD alternans in excess of 10 ms was defined as the APD alternans threshold.

Ca^{2+} Imaging in Myocytes

For current-clamp experiments the external solution contained in mM: 140 NaCl, 5.4 KCl, 2.0 $CaCl_2$, 0.5 $MgCl_2$, 10 HEPES, and 5.6 glucose (pH 7.4). Patch pipettes were filled with a solution that contained in mM: 90 K-aspartate, 50 KCl, 5 MgATP, 5 NaCl, 1 $MgCl_2$, 0.1 Tris GTP, 10 HEPES, and 0.1 Fluo-3 K-salt (Molecular Probes, OR) (pH 7.2). For the voltage-clamp experiments the external solution contained in mM: 140 NaCl, 5.4 CsCl, 2.0 $CaCl_2$, 0.5 $MgCl_2$, 10 HEPES, and 5.6 glucose (pH 7.4). Patch pipettes were filled with a solution that contained in mM: 123 CsCl, 20 TEACl, 5 MgATP, 5 NaCl, 1 $MgCl_2$, 0.1 Tris GTP, 10 HEPES, and 0.1 Rhod-2 K-salt (Molecular Probes, OR) or 0.1 Fluo-3 K-salt (pH 7.2).

To record simultaneously intra SR and cytosolic Ca^{2+} levels , Fluo-5N and Rhod-2 were excited by 488- and 543-nm laser lines, and fluorescence was acquired at wavelengths of 500–530 and >590 nm, respectively. In these experiments10 mM 2,3-butanedione monoxime (BDM) was used to prevent movement artifacts.

Ca^{2+} currents (I_{Ca}) and corresponding Ca^{2+} transients were evoked by 300 ms depolarization steps from a holding potential of -50 to 0 mV, unless otherwise stated. Amplitude of Ca^{2+} alternans was defined as 100–(A2/A1)*100 (%), where A1 and A2 are amplitudes of two consecutive cytosolic Ca^{2+} transients.

Intra-SR Ca^{2+} dynamics in Fluo-5N loaded saponin-permeabilized myocytes were studied using an intracellular solution that contained: (mM) 120 K-aspartate, 20 KCl, 3 MgATP, 0.81 $MgCl_2$, 10 phosphocreatine, 5 U ml^{-1} creatine phosphokinase, 0.5 EGTA (pCa 7) and 20 HEPES (pH 7.2). Kinetics of SR Ca^{2+} uptake were measured in the presence 30 μM ruthenium red, a RyR inhibitor.

The temporal dynamics in Fluo-5N fluorescence in permeabilzed myocytes were expressed as $\Delta F_{CAFF}/\Delta F_{MAX} = (F–F_{CAFF})/(F_{MAX}–F_{CAFF})$, where F represents fluorescence at time *t*, F_{CAFF} represents the fluorescence level of the cells after the application of 10 mM of caffeine, F_{MAX} represents Fluo-5N fluorescence in the presence of 10 mM of [Ca^{2+}]. Fluo-5N fluorescence in voltage-clamped myocytes was converted to [Ca^{2+}] according to the following equation: $[Ca^{2+}]=Kd*(F–F_{CAFF})/(F_{MAX}–F)$, where Kd was 400 μM.[6] F_{MAX} was determined by application of 10 mM of [Ca^{2+}] and 0.1% saponin in the presence of 10 mM of BDM.

Measurements of Reactive Oxygen Species (ROS) Production and Ryanodine Receptor (RyR) Free Thiol Content

To measure ROS production, cells were loaded with 10 μM 5-(and-6) chloromethyl-2',7'-dichlorodihydrofluoroscein diacetate (DCFDA) for 30 min at room temperature, followed by 15 min for de-esterification. Loaded cells were excited with the 488 nm line of argon laser and emission was collected at 500-560 nm using Olympus Fluoview 1000 confocal system.

mBB fluorescence in SR samples was measured using EpichemiII Darkroom fluorescence system (UVP

Bioimaging Sys., CA; excitation 365 nm and emission 400-600 nm). Images were acquired and analyzed using LabWorks 4.0 software.

Reagents.
All reagents are from Sigma-Aldrich, Inc (MO), unless otherwise indicated.

RESULTS

The effects of β-adrenergic agonist Iso on I_{Ca} and Ca^{2+} transients were not altered in VF myocytes when compared to control (Figure S1). In the absence of Iso, average peak I_{Ca} density was -2.9±0.6 and -3.0±0.4 pA/pF in control (n=7) and in VF (n=7) myocytes, respectively. In the presence of 100 nM Iso, the average peak I_{Ca} density was -8.3±1.0 (n=9) in control and -9.3±1.0 pA/pF (n=7) in VF myocytes, respectively. These values are similar to those reported by other groups for canine ventricular myocytes.[7,8] In the absence of Iso, average amplitude of Ca^{2+} transients was 1.0±0.2 and 0.9±0.1 ($\Delta F/F_0$) in control (n=7) and VF (n=7) myocytes, respectively. In the presence of Iso, average amplitude of Ca^{2+} transients was 2.8±0.3 and 2.9±0.3 ($\Delta F/F_0$) in control (n=9) and VF (n=7) myocytes, respectively.

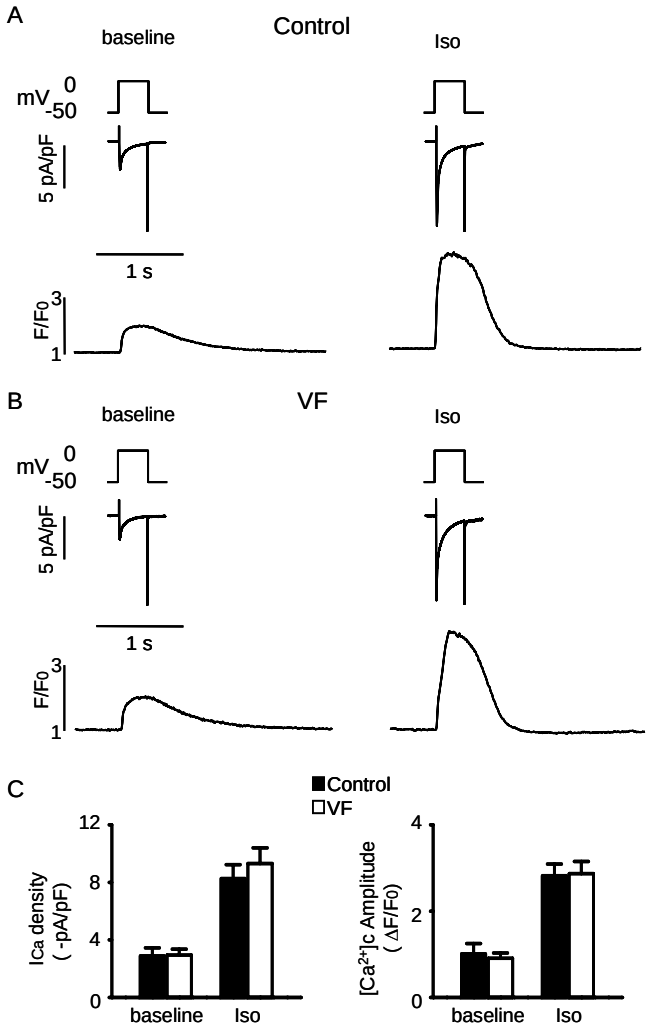

Figure S1. Isoproterenol (Iso) produced similar effects on I_{Ca} and Ca^{2+} transients in control and VF myocytes. Representative I_{Ca} and corresponding temporal profiles of Fluo-3 fluorescence recorded in control (A) and in VF (B) myocytes before and during application of 100 nM Iso. Upper traces show voltage protocol used. C, Average values of the peak I_{Ca} density and amplitude of Ca^{2+} transients recorded in the absence and presence of 100 nM Iso in control and VF myocytes, respectively.

Parameter	Control (n=7)	VF (n=8)
I_{Ca} density (pA/pF)	-9.3±1.1	-9.0±1.2
τ_1 (ms)	13.0±0.9	13.8±2.2
τ_2 (ms)	72.8±2.6	90.3±14.0

Online Table I. Parameters of I_{Ca} recorded at 0 mV as described in Figure 3 of the main manuscript. τ_1 and τ_2 are time constants of exponential fit of the decay of I_{Ca}

As shown in online Table I, the amplitude and decay of I_{Ca} evoked by 300 ms step depolarization from -50 to 0 mV, were not different in control and VF myocytes.

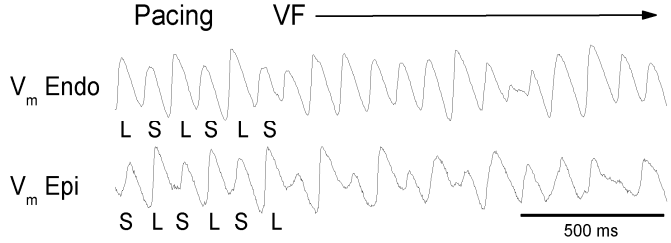

Figure S2. Spatially discordant alternans preceding the induction of ventricular fibrillation (VF) in a left ventricular wedge preparation from a VF canine. Shown are action potential traces from the endocardium (Endo) and epicardium (Epi) during pacing induced alternans and the initiation of VF. Note that preceding VF, the short-long pattern of action potential duration alternans at the Endo (LSLSLS) is spatially out-of-phase with that from the Epi (SLSLSL).

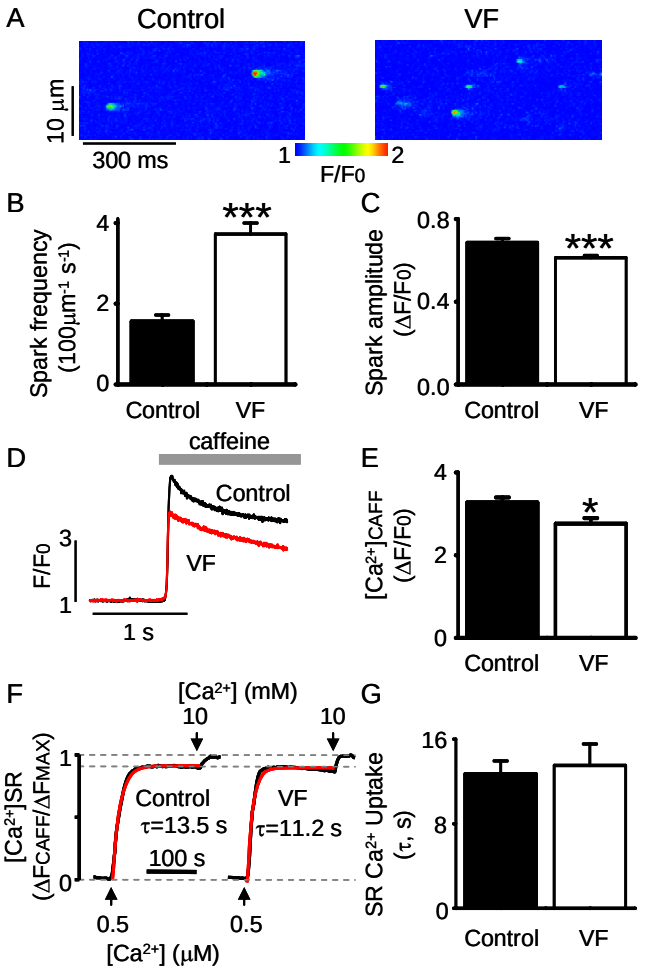

Figure S3. VF myocytes display increased diastolic SR Ca^{2+} leak and conserved SERCA function. A, Representative line-scan images of Ca^{2+} sparks in control and VF cells. B, Average values of spark frequency were 1.57±0.16 (n=52) and 3.72±0.27 (n=41, 100 $\mu m^{-1}s^{-1}$) in control and VF cells, respectively. ***P<0.001, vs. control. C, Average amplitudes of Ca^{2+} sparks were 0.69±0.02 (n=212) and 0.61±0.01 (n=412, $\Delta F/F_0$) in control and VF cells, respectively. ***P<0.001, vs. control. D, Temporal profiles of line-scan images of caffeine-induced Ca^{2+} transients in control and VF cells. E, Average amplitudes of caffeine-induced Ca^{2+} transients ($[Ca^{2+}]_{CAFF}$) were 3.27±0.12 (n=11) and 2.76±0.14 (n=12, $\Delta F/F_0$) in control and VF cells, respectively. *P<0.05, vs. control. F, Time course of SR Ca^{2+} uptake in permeabilized control and VF myocytes, measured with Fluo-5N-loaded SR in the presence of 30 μM ruthenium red. The SR Ca^{2+} uptake was initiated by the addition of 0.5 μM of Ca^{2+}. G, Average time constants (from exponential fit) of SR Ca^{2+} uptake were 12.7 ± 1.2 s in control (n=10) and 13.5 ± 2.0 s in VF myocytes (n=9).

Figure S2 demonstrates an example of APD alternans preceding VF observed in a wedge preparation from a VF susceptible dog.

Figure S3 shows that permeabilzed VF myocytes display increased Ca^{2+} sparks frequency (panels A and B), decreased SR Ca^{2+} load (panels D and E) and conserved SERCA function (panels F and G). NCX activity was assessed by measuring the rate of decay of caffeine-induced Ca^{2+} transients in control and VF voltage-clamped myocytes. As demonstrated in Figure S4, the rate of decay of caffeine-induced Ca^{2+} transients was not different between control and VF myocytes.

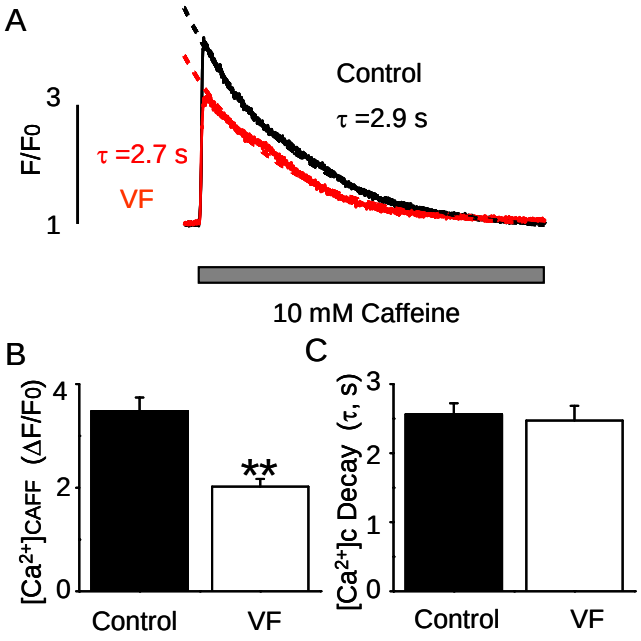

Figure S4. The rate of cytosolic Ca^{2+} removal by NCX is not altered in VF myocytes. (*A*) Representative recordings of Ca^{2+} transients induced by rapid application of 10 mM caffeine in control and VF myocytes. (*B*) Average amplitudes (ΔF/F0) of caffeine-induced Ca^{2+} transients were 3.5±0.3 (n=7) in control and 2.0±0.2 (n=7) in VF myocytes, respectively. (*C*) The decay time constants of the caffeine-induced Ca^{2+} transients in control (n=6) and HF (n=6) myocytes were 2.6±0.2 and 2.5±0.2 s, respectively. (** P<0.01).

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