Supplemental Material

Contraction and whole Ca^{2+} transients in isolated mouse ventricular myocytes.

Wild type 2-4 month old mice were anesthetized with intraperitoneal pentobarbital sodium (100 mg/kg/ip). To assess for sarcomere shortening, cells were imaged using field stimulation (0.5 Hz) in an inverted fluorescence microscope (Diaphot 200; Nikon, Inc). Sarcomere length was measured by real-time Fourier transform (IonOptix MyoCam, CCCD100M).Twitch amplitude is expressed as a percentage of resting cell length. Twitch kinetics was quantified by measuring the time to peak shortening and the time from peak shortening to 50% relaxation. For whole Ca^{2+} transient measurements, myocytes were loaded with the Ca^{2+} indicator fluo-4/AM (Molecular Probes, 20 μM for 30 min) and Ca^{2+} transients were measured under field-stimulation (0.5 Hz) in perfusion solution by confocal laser scanning microscope (LSM510, Carl Zeiss). Digital image analysis used customer-designed programs coded in Interactive Data Language (IDL).

Whole Ca^{2+} transients and SR Ca^{2+} load in isolated rat ventricular myocytes.

Isolation of rat ventricular myocytes was carried as follows. The enzyme used for tissue dissociation was Liberase Blendzyme 3 or 4 (13-20 Wuensch Units/Heart) sometimes supplemented with 5-10 Units of Dispase II (both Roche Diagnostics, Indianapolis, IN). Ventricular myocytes were then plated onto superfusion chambers, with the glass bottoms treated with natural mouse laminin (Invitrogen, Carlsbad, CA). The standard Tyrode's solution used in all experiments contained (in mM): NaCl 140, KCl 4, $MgCl_2$ 1, glucose 10, HEPES 5, and $CaCl_2$ 1, pH 7.4. Myocytes were loaded with 6 μM Indo-1/AM for 25 min and subsequently perfused for at least 30 min to allow for deesterfication of the dye. Some cells were pretreated with 0.5 mM AS (in some Caffeine experiments with 1 mM), washed and then loaded with Indo-1/AM. Concentration of the AS stock solution

was verified by absorbance at 250 nm. All experiments were done at room temperature (23–25°C) using field stimulation. Ca^{2+}-transients were recorded with Clampex 8.0 and data analyzed with Clampfit.

FRET analysis

Cells were transfected with a FRET-based sensor for cAMP and imaged 48 hrs after transfection. Cells were continuously perfused with HEPES buffered Ringer's modified saline (1 mmol $CaCl_2$/liter) at room temperature. Cells were imaged on an inverted Olympus IX50 microscope upon excitation at 430 nm. Images analysis was performed by using ImageJ (Rasband, W.S., ImageJ, NIH, Bethesda, Maryland, USA). FRET values were measured as the 480/535nm emission ratio intensity (R) and were normalized to the 480nm/535nm value at time 0s (R_0).

Fluorescent probes for two-photon laser scanning microscopy and image acquisition.

The cationic potentiometric fluorescent dye tetramethylrhodamine methyl ester (TMRM) was used to monitor changes in $\Delta\Psi_m$. The production of the fluorescent glutathione adduct GSB from the reaction of cell permeant monochlorobimane (MCB) with reduced glutathione (GSH), catalyzed by glutathione S-transferase, was used to measure intracellular glutathione levels. Details of GSH measurements are provided in the on-line supplement. The dish containing the cardiomyocytes was equilibrated at 37°C with unrestricted access to atmospheric O_2 on the stage of a Nikon E600FN upright microscope. Then, cells were loaded with 100 nM TMRM and 50 μM MCB for at least 20 min. Images were recorded using a two photon laser scanning microscope (Bio-Rad MRC-1024MP) with excitation at 740nm (Tsunami Ti:Sa laser, Spectra-Physics). The red emission of TMRE was collected at 605±25nm and the blue fluorescence of GSB

was collected at its maximal emission (480nm). Images were analyzed offline using ImageJ software (Wayne Rasband, NIH).

***RyR2 single channel recordings in planar lipid bilayers*.**

Briefly, a phospholipid bilayer of PE:PS (1:1 dissolved in n-decane to 20 mg/ml) was formed across an aperture of ~300 μm diameter in a delrin cup. The cis chamber (900 μl) was the voltage control side connected to the head stage of a 200A Axopatch amplifier, while the trans chamber (800 μl) was held at virtual ground. Both chambers were initially filled with 50 mM cesium methanesulfonate and 10 mM Tris/Hepes pH 7.2. After bilayer formation, cesium methanesulfonate was raised to 300 mM in the cis side and 100 to 200 μg of mouse cardiac SR vesicles was added. After detection of channel openings, Cs^+ in the *trans* chamber was raised to 300 mM to collapse the chemical gradient. Single channel data were collected at steady voltages (-30 mV) for 2-5 min. Channel activity was recorded with a 16-bit VCR-based acquisition and storage system at a 10 kHz sampling rate. Signals were analyzed after filtering with an 8-pole Bessel filter at a sampling frequency of 1.5-2 kHz. Data acquisition and analysis were done with Axon Instruments software and hardware (pClamp v8.0, Digidata 200 AD/DA interface).

Preparation of crude SR vesicles from murine hearts.Measurements of ATP-dependent Ca^{2+} uptake by murine cardiac SR vesicles.

Pooled hearts from C57 mice were placed in 0.9% saline on ice, trimmed of atrial and connective tissue, and weighed. The finely minced heart muscle was homogenized in 10 mM $NaHCO_3$ using a Polytron blender and the SR vesicles were separated from the myofilaments, mitochondria and nuclear membranes by differential centrifugation at 8,500 and 45,000 x *g*. SR vesicles suspended in 0.25 M sucrose + 10 mM MOPS, pH 7.0 were frozen and stored in liquid nitrogen prior to use. Twenty minutes prior to

measuring Ca^{2+} uptake, cardiac SR vesicles (1 mg/ml in storage buffer) were incubated with 250 μM AS. After dilution of the SR membranes in the Ca^{2+} uptake buffer, changes in kinetic behavior due to AS/HNO were seen after a delay of ~15 min and remained in effect for the duration of the experiment (45-60 min).