Online Supplemental Methods Section

Canine Pacing-Induced Tachycardia Heart Failure Model

Induction of heart failure was carried out as described previously[1], using protocols approved by the Institution's Animal Care and Use Committee. In brief, a VVI pacemaker (Medtronics) was implanted in mongrel dogs of either sex. Pacing at 240 bpm was maintained for 3-4 weeks, during which time the animals developed typical symptoms of heart failure including lethargy, loss of appetite, ascites, etc. Hemodynamic decompensation was confirmed by recording left ventricular (LV) pressure waveforms (under anesthesia with 25 mg/Kg tiopental) prior to sacrifice using a micromanometer - tipped LV catheter inserted through the right femoral artery. An increased end-diastolic pressure (>20mmHg), slowed rate of pressure rise and slowed relaxation rate were evident in F[2].

Isolation of midmyocardial cardiomyocytes

After left lateral thoracotomy, the heart was perfused with ice-cold cardioplegic solution, containing (mmol/L): KCl 104; NaCl 32; $NaHCO_3$ 10, taurine 10, BDM (butanedione monoxime) 20, pH 7.4, and quickly excised. The region of the left ventricular free wall perfused by the left anterior descending coronary artery was excised, cannulated and perfused at 12 ml/min. The basic Ca^{2+}-free isolation solution contained[3], in mmol/L : NaCl 130; KCl 4.5; $MgCl_2$ 5; HEPES 23; glucose 21; taurine 20; creatine 5; NaH_2PO_4 1; Na pyruvate 5; pH 7.25 (titrated with NaOH), at 37^{o}C, oxygenated with 100% O_2. The cardiac muscle was perfused in sequence[4] with: 1) isolation solution with added 8 mmol/L EGTA for 15 min.; 2) isolation solution with 50 μmol/L Ca, 1 mg/ml collagenase (type I, 255 U/mg, Worthington Biochemical Corp., Freehold, N. J.) and 0.1 mg/ml protease (type XIV, Sigma Chemical Co., St. Louis, MO) for 12 min., and 3) isolation solution containing 100 μmol/L Ca and 10 mmol/L BDM for 6 min. for washout. Chunks of well-digested ventricular tissue from the midmyocardial layer of the ventricle were dissected out (after removing the epicardial and endocardial layers) and cells were mechanically disaggregated, filtered through nylon mesh and stored in modified Tyrode's solution containing 1 mmol/L Ca. The procedure yielded Ca^{2+}-tolerant quiescent myocytes which survived well for up to 8 hours.

Single-cell electrophysiology studies

Cells were placed in a heated chamber on the stage of an inverted fluorescence microscope (IX70, Olympus, Inc.) and superfused with a physiological salt solution (see Methods). All experiments were carried out at 37 ^{o}C. We used a custom-built heated device capable of rapidly changing the external solution around the cell, while keeping the temperature at 37 ^{o}C. Borosilicate glass pipettes of 1-4 MΩ tip resistance were used for whole-cell recording with an Axopatch 1D amplifier coupled to a Digidata 1200A personal computer interface (Axon Instruments, Foster City, CA) using custom-written software.

$[Ca^{2+}]_i$ measurement

$[Ca^{2+}]_i$ measurement was performed as described previously[4] using the K^+ salt form of indo-1. Cellular autofluorescence was recorded before rupturing the cell-attached patch and subtracted prior to determining R (ratio of 405nm emission/495nm emission). Ca_i was calculated according to the equation $Ca_i = K_d \times \beta \times [(R - R_{min})/(R_{max} - R)]$[5], using a K_d of 844 nmol/L[6], and experimentally determined $R_{min} = 1$, $R_{max} = 4$ and $\beta = 2$.

The main experimental protocol

After establishing the whole cell mode, and capacitance compensation, cells were paced at a slow rate (0.1 Hz) until the indo-1 signals reached steady-state (usually 2-7 min), signifying sufficient diffusion of the pipette solution into the cell. The experimental protocol was then started and consisted of a train of 0.5 sec depolarizations from -80mV to +10mV, applied at 0.5 Hz until the $[Ca^{2+}]_i$ transients reached steady-state. At this point 30 μmol/L tetrodotoxin (Na^+ channel blocker) was rapidly applied for a few beats, to allow a better estimation of the peak of the L-type Ca^{2+} current ($I_{Ca,L}$). The short TTX application did not affect the amplitude and rate of rise of the $[Ca^{2+}]_i$ transients. TTX was then washed off, the train of depolarization stopped and caffeine was rapidly applied to measure sarcoplasmic reticulum Ca^{2+} load (Ca_{SR}).

During caffeine application, $[Ca^{2+}]_i$ rose rapidly and then decayed exponentially, extruded (mainly) by NCX, which generated an inward current (Fig 1). In some experiments, 10 sec after caffeine wash-off, caffeine was reapplied. The second caffeine application produced no change in $[Ca^{2+}]_i$, reassuring that all SR Ca^{2+} was released with the first application. The total Ca^{2+} load of the SR was estimated from the integral of the NCX

current (?I_{NCX}), which represented the charge moved by NCX while extruding Ca^{2+}. ?I_{NCX} was further divided by the elementary charge (1.6×10^{-19} C) and Avogadro's number (6.023×10^{23}), giving the number of moles of Ca^{2+} extruded. Cell volume was estimated from cell capacitance, divided by the membrane specific capacitance (1 $\mu F/cm^2$) and a typical cell surface/volume ratio of 0.5 μm^{-1} (cf. ref.[7]). Cytosolic (non-mitochondrial) volume is 65% of cell volume. In the end, we used the equation Ca_{SR} (μmol/L) = 76 x ?I_{NCX} (pC) / cell capacitance (pF). The factor "76" combines all the constants used. Please note that the unit (μmol/L) refers to μmoles Ca^{2+} stored in the SR divided by the *total* cell volume (in L). Consistent with previous studies[1,2,4], cell capacitance and therefore the calculated cell volume were similar in F and N cells.

References

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