Animal Model

Left ventricular hypertrophy was induced by methods described previously in detail\(^1\). Briefly, a fixed size (2.8-3.0 mm in diameter) constricting band was placed around the ascending aorta of young cats (~1.0 kg). Previously we have shown that as animals grow they develop either a concentric, compensated hypertrophy or a hypertrophy with dilatation and signs and symptoms (dyspnea, ascites) of heart failure\(^2\).

The classification of aortic-banded animals into compensated LVH and CHF groups was determined by echocardiography, physical examination and the presence of ascites and/or pleural effusions at sacrifice. Banded (n = 10) and control cats (n = 5) were sedated with ketamine (15-20 mg/kg). Using a Sonos 5500 (Hewlett Packard, Andover, MA) echocardiography system, m-mode images of the LV were recorded and chamber geometry and wall thickness were measured. Fractional shortening was derived from m-mode images and expressed as \[
\frac{[(\text{diastolic chamber diameter} - \text{systolic chamber diameter}) \times 100\%]}{\text{diastolic chamber diameter}}\].

Myocyte Isolation

Left ventricular myocytes were isolated from normal and hypertrophied hearts using techniques previously described by our laboratory\(^3\). Cells were maintained at room temperature (20-22\(^\circ\)C) with a 95% O\(_2\) and 5% CO\(_2\) overlay until used.

Experimental Procedures

Myocytes were loaded with 5-10 µM Fluo-4 AM (Molecular Probes) for 15-30 minutes. Experiments were performed on an inverted Nikon Diaphot microscope coupled to a Bio-Rad MRC-600 confocal line scanning system. Images were acquired using a Nikon PlanApo 40X oil immersion lens (N.A. 1.3). Myocytes were only studied if their length was ±10 degrees of the longitudinal axis of the image plane. We also imaged a stimuli designed by the authors to align the line-scan images for analysis purposes. In all experiments, cells were superfused with a modified Tyrode solution containing (in mM): 150 NaCl, 5.4 KCl, 1.2 MgCl\(_2\), 10 glucose, 2.0 Na-pyruvate, 1 mM CaCl\(_2\) and 5 HEPES, pH 7.4. All experiments were performed at 32\(^\circ\)C.
For fluorescence measurements, the F₀ (or F unstimulated), the average fluorescence (F) of the cell 50 msec prior to stimulation was measured. Data were collected with Comos software (BioRad) and analyzed off-line by IDL 5.5 (Research Systems Inc.) and Clampfit 8.0 (Axon Instruments). A K₅ of 1100 for Fluo-4 Ca²⁺ binding, and resting [Ca²⁺]ᵢ of 150 nM were used in all calculations. The maximal Fluo-4 fluorescence (FMAX/F₀) was determined by damaging the cell with a microelectrode to induce a large Ca²⁺ influx as described by Trafford et al⁴. F_MAX values greater than 8 were routinely obtained, documenting that the values reported here are within the appropriate linear range of the indicator.

Electrophysiological experiments were performed using the whole-cell patch clamp technique. Micropipettes (2-4 MΩ) were filled with physiological filling solution (in mM: 10 HEPES, 130 K-aspartate, 5 K-ATP, 20 KCl, 10 NaCl, and 1 MgCl₂(H₂O)₆, pH=7.2). Membrane voltage was controlled by an Axoclamp 2 voltage-clamp amplifier in discontinuous mode (7 kHz) (Axon Instruments). All voltage clamp test steps were preceded by five conditioning pulses (-70 to +10 for 250 msec) at 1.0 Hz to normalize SR Ca²⁺ load. The amplifier was controlled by pCLAMP 8.0 software and data were acquired by a Digidata 1320. Data were analyzed off-line by Clampfit 8.0 software (Axon Instruments).

L-type Ca²⁺ current was measured in Na⁺ and Ca²⁺ free bath and pipette solutions to eliminate overlapping currents, as described previously⁵. The difference between the peak inward and steady state currents were measured at each test potential. All currents were normalized by the cell input capacitance.

An analysis similar to that described by Heinzel et al⁶ was used to characterize the synchrony of SR Ca²⁺ release. Spatial Fluo-4 fluorescence profiles were analyzed at specific times after the stimulus. The percentage of the line scan with a fluorescence (F) above 50% of the peak value obtained during that Ca²⁺ transient (F₅₀) was determined at selected time points (every 10 msec up to 60 msec). A larger percentage of a spatial profile with an F over F₅₀ constituted a greater amount of synchronous SR Ca²⁺ release.

Data are expressed as means ± SEM. Unpaired student t-tests were used where appropriate. One-way ANOVA was used for echocardiography and whole cell
fluorescence statistics. Two-way ANOVA was used for the effects of synchrony and time. A $p \leq 0.05$ was considered significant.

1. Bailey BA, Houser SR. Calcium transients in feline left ventricular myocytes with hypertrophy induced by slow progressive pressure overload. *J Mol Cell Cardiol*. 1992;24:365-73.