Effects of redox environment on calcium alternans in isolated rabbit cardiomyocytes

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Cardiac alternans is a multifactorial phenomenon linked to cardiac arrythmias. At the cellular level cardiac alternans is defined by beat-to-beat alternations in contraction amplitude (mechanical alternans), action potential duration (electrical or action potential duration alternans) and Ca transient amplitude (Ca alternans) at constant stimulation frequency. The aim of this project was to characterize the effect of changes in the cellular redox environment on Ca alternans in cardiac myocytes.

Single myocytes (from New Zealand White rabbits) were isolated enzymatically by retrograde Langendorff perfusion. Ca alternans were induced by incrementally increasing the pacing frequency (electrical field stimulation) until stable Ca alternans occurred. The frequency at which stable Ca alternans were observed varied from cell to cell and ranged from >1 to 2.5 Hz at room temperature. Global cytosolic Ca transients were measured with Indo-1. In some experiments, cytosolic Ca alternans and intra-SR Ca alternans were simultaneously measured with the fluorescent Ca indicators Rhod-2 and Fluo-5N, respectively. Confocal microscopy was used to measure Ca sparks with Fluo-4.

Reducing agents dithiothreitol and reduced glutathione partially abolished Ca and mechanical alternans by restoring diastolic Ca and Ca transient amplitudes. A decreased sarcoplasmic reticulum (SR) Ca release flux but not Ca content, together with a decreased Ca spark frequency, suggest that reducing agents normalized alternans through effects on the SR Ca release channel (ryanodine receptor type-2). Addition of a membrane permeant superoxide dismutase mimetic, Tempol, had little effect on Ca alternans, suggesting the possible role of dithiotreitol directly acting on the ryanodine receptor. These data highlight that the redox state of the cell may be important in the generation of Ca and mechanical alternans during oxidative stress.