Poster #31
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Title of Research: Physiologic and Pathologic Consequences of Inhibiting Cardiac MCU
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Introduction/Purpose:
Mitochondrial Ca2+ is a secondary messenger necessary to increase the activity of at least 3 matrix dehydrogenases that enhance oxidative phosphorylation and match cellular energy supply with demand. At the same time, mitochondrial Ca2+ overload causes mitochondrial dysfunction, loss of mitochondrial membrane potential ($m\Delta\psi$) and myocyte death. Mitochondrial Ca2+ overload is a feature of ischemia-reperfusion (I/R) injury, a prevalent health condition in aging populations, but there are no therapies designed to inhibit mitochondrial Ca2+ overload. The molecular identity of the mitochondrial calcium uniporter (MCU) was recently identified, allowing us to develop a novel transgenic mouse with cardiac delimited inhibition of MCU current by transgenic expression of a dominant negative MCU (DN-MCU). We hypothesized that DN-MCU mice would survive normally into adulthood, but would show physiologic changes consistent with an ATP deficient state and protection from cell death.

Experimental Design:
The αMHC promoter was used to drive and restrict expression of DN-MCU to cardiomyocytes. Fluorescent, patch clamp and absorbance assays were used to measure mitochondrial Ca2+ uptake in DN-MCU mitochondria. A hexokinase trap and NMR analysis measured Ca2+-induced ATP production from isolated mitochondria. Whole hearts were perfused on a Langendorff system for all I/R studies. Hearts were loaded with TMRM and imaged on a confocal microscope for in situ measurement of $m\Delta\psi$ during I/R. TUNEL and TTC stains were used to measure cell death and viable myocardium, respectively. Whole heart homogenates were used for western blotting. Cardiac MRI was used to measure cardiac function and morphology.

Results:
DN-MCU mice were viable and cardiac mitochondria showed total inhibition of IMCU. Peak ATP production was not altered in DN-MCU mitochondria, but DN-MCU mitochondria showed protection from dissipation of $m\Delta\psi$, O2 consumption and ATP production after supraphysiologic Ca2+ insult. Unexpectedly, in situ measurement of $m\Delta\psi$ showed a steep decline in DN-MCU hearts at onset of ischemia without recovery during reperfusion. Despite a loss of $m\Delta\psi$, DN-MCU hearts showed less cell death and more viable myocardium after I/R. DN-MCU hearts show a highly penetrant phenotype of right ventricular dilatation with preserved left ventricular morphology and function.

Conclusions:
We conclude that inhibition of cardiac IMCU protects hearts from I/R injury, but therapeutic potential is limited by morphologic changes consistent with dilated cardiomyopathy.