Metabolic Syndrome and SIRT1 Mutation Impair Ca^{2+} Channels in Coronary Smooth Muscle Cells of Ossabaw Miniature Swine

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Background:
Changes in Ca^{2+} regulation have been implicated in various pathologies such as coronary artery disease and metabolic syndrome (MetS), thereby potentiating these diseases. Our lab has shown that MetS decreases voltage-gated Ca^{2+} channel (VGCC) activity and sarcoplasmic reticulum (SR) Ca^{2+} release in coronary smooth muscle cells and increases coronary artery disease in Ossabaw miniature swine. Furthermore, decreased SIRT1 enzyme function can impair Ca^{2+} signaling and increase coronary disease and MetS. We hypothesized that impaired SIRT1 and MetS would decrease VGCC function and SR calcium store.

Methods:
CRISPR/Cas9 methods delivered a leucine to proline point mutation in SIRT1 ($SIRT1^{L100P}$) into the Ossabaw swine genome to compare to wild type (WT), mimicking the naturally occurring mutation in humans which decreases SIRT1 activity. Four treatment groups of juvenile swine were based on genotype and diet: WT Lean, SIRT1 Lean, WT MetS, and SIRT1 MetS. Lean swine were fed normal chow and MetS were fed a hypercaloric, atherogenic diet for 7 months. The left anterior descending coronary artery was harvested and enzymatically digested to obtain cells. Fluorescence microscopy measured the Ca^{2+} indicator fura-2 in single cells. Depolarization of cells with perfusion of 80 mM K^+ was used to elicit Ca^{2+} influx through VGCC. Caffeine (5 mM) exposure activated the Ca^{2+} release channel (ryanodine receptor) on the SR.

Results:
MetS was confirmed by increased body weight, impaired glucose tolerance, hyperinsulinemia, and hypercholesterolemia. Coronary atherosclerosis was shown by angiography, intravascular ultrasound, and gross imaging. A two-way analysis of variance revealed statistically significant overall effects of genotype ($p=0.02$), diet ($p<0.0001$), and an interaction ($p<0.0001$) between these variables to decrease VGCC function. In contrast, no effect was observed on SR Ca^{2+} release.

Conclusion and Potential Impact:
SIRT1 inhibition and MetS decreased VGCC function independently, but not additively or synergistically. (Support: NIH T35HL110854, DK120240, DK09751.)