SIRT1 Inhibition Impairs Ca^{2+} Buffering in Coronary Smooth Muscle Cells of Ossabaw Miniature Swine

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Background: SIRT1 is a deacetylase that has diverse roles in intracellular Ca^{2+} signaling, metabolism, and cardiovascular disease. SIRT1 increases sarco-endoplasmic reticulum Ca^{2+} ATPase (SERCA) activity that is essential to buffer the increase in Ca^{2+} induced by release from the sarcoplasmic reticulum (SR). Our lab has shown that metabolic syndrome (MetS) impairs SERCA activity in coronary smooth muscle cells and causes coronary artery disease in Ossabaw miniature swine. We hypothesized that SIRT1 inhibition and MetS would impair Ca^{2+} buffering.

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) and mimic the naturally occurring mutation in humans and decrease 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. The cells were exposed to 5 mM caffeine to maximally release stores of Ca^{2+} from the SR. Ca^{2+} buffering capacity of each cell was analyzed after the caffeine-induced peak increase to assess Ca^{2+} efflux and SERCA activity.

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. The rapid phase of Ca^{2+} buffering due to Ca^{2+} efflux was not affected by SIRT1 mutation or MetS. The slower phase of Ca^{2+} buffering due to SERCA activity was impaired only by SIRT1 mutation ($p<0.0005$), not by MetS.

Conclusion: SIRT1 mutation alone inhibited SERCA buffering of Ca^{2+} in coronary smooth muscle. (Support: NIH T35HL110854, DK120240, DK09751.)