MUTATIONS IN CPVT1-ASSOCIATED AND CPVT1-UNASSOCIATED RYR2 CALCIUM BINDING RESIDUE REVEAL REMODELING OF EC-COUPLING IN HIPSC-CMS

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Reports on 3-dimentional near-atomic Cryo-EM images of skeletal ryanodine receptor have revealed identity and location of Ca^{2+} and Caffeine binding sites residues. Mutating RyR2 Ca^{2+}-binding residues in homologous HEK cell, though revealing the roles of binding residues on RyR2 function, fail to account for possible remodeling of other cellular Ca^{2+}-signaling pathways, likely to occur in diseased states. The remarkable similarity of Ca^{2+}-signaling in human stem-cell derived cardiomyocytes (hiPSC-CMs) to adult cardiomyocytes led us to examine the effects of CPVT1-associated or CPVT1-unassociated mutations of the putative RyR2 calcium binding site residues on EC-coupling consequences of hiPSC-CMs Ca^{2+}-signaling. We introduced Q3925E mutation associated and E3848A mutation unassociated with CPVT1 in hiPSC-CMs using CRISPR/Cas9 gene editing and determined their EC-coupling phenotypes.
In TIRF-imaged and voltage-clamped WT and mutant hiPSC-CMs infected with SR-targeted ER-GCaMP6 probe: 1) I_{Ca} densities were comparable (7-10pA/pF) in mutant- and WT-cells; 2) I_{Ca-} and caffeine-triggered Fura-2 (cytosolic calcium) or ER-GCaMP6 (SR Ca^{2+} release) signals were significantly suppressed in both mutants; 3) Arrhythmic Fura-2 signals in either mutant were not accompanied by ER-GCaMP6 Ca-release signals; 4) Even though caffeine failed to trigger Ca^{2+} release in mutant voltage-clamped cells, only ~20% to ~70% of cells responded respectively to 5 & 20mM caffeine in intact cells, but these responses were delayed, slow, and 2-APB- or ruthenium red-sensitive. Mutations of RyR2 Ca^{2+}-binding residues, irrespective of CPVT1 association, reveal interaction between Ca^{2+} and caffeine binding-sites and unmask remodeling of EC-coupling in heart cells that accounts for arrhythmogenic Ca-transients of mutant cells.

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