PO-616-02

HETEROGENEOUS CAMP SIGNALING IN THE INTACT HEART IS SEX DEPENDENT

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Background: cAMP is key for transducing autonomic signals into downstream electrophysiological responses. Previous studies have shown intracellular heterogeneity and compartmentalization of cAMP signaling. Yet, if cAMP signaling occurs heterogeneously throughout the intact heart, and how this translates into functional responses, has not been explored.

Objective: To determine the spatiotemporal kinetics of cAMP signaling in the intact heart and the underlying mechanisms responsible.

Methods: Male and female cardiac-specific CAMPER reporter mice that report cAMP binding by changes in FRET were used in 12 weeks. Hearts were excised and Langendorff-perfused into downstream electrophysiological responses. Previous studies have shown simultaneous cAMP and Vm imaging on a novel integrated system.

Results: In male hearts, cAMP was uniformly activated in response to β-AR stimulation with bolus norepinephrine (NE, 1.5 μM). Conversely, in female hearts NE led to a greater change in cAMP activity in basal regions vs. the apex (n=5, p<0.05). Moreover, cAMP deactivation was slower in the base vs. apex, in female (n=6, p<0.01) but not male hearts (n=6). Apex-base differences were also evident following PDE inhibition with IBMX (100 μM), with a greater change in cAMP activity in the apex vs. base in both female (n=7, p<0.001) and male hearts (n=5, p<0.05). Likewise, PDE activity assays showed higher total PDE activity in apical regions (n=10, p<0.01), with more apical PDE activity in female vs. male hearts (n=5, p<0.05). In female hearts, faster apical cAMP deactivation following bolus NE was associated with a significant difference in action potential duration (APD90) between apex and base (n=3, p<0.05), but APD90 was not significantly different between regions in male hearts.

Conclusion: Using novel whole heart imaging, we have shown female hearts display lower maximal cAMP activity and faster deactivation in the apex, in part, due to elevated PDE activity in this region. This heterogeneity was not observed in male hearts. These findings may have important implications for electrophysiological responses regulated by the cAMP pathway, particularly in heart failure, where PDE activity is altered.

PO-616-03

HIGH-THROUGHPUT SCREENING TO IDENTIFY DRUGS THAT CAN TREAT LONG QT SYNDROME CAUSED BY TRAFFICKING-DEFICIENT K$_{r}$11.1 (HERG) VARIANTS

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Background: The potassium channel K$_{r}$11.1 plays an important role in repolarization of cardiac action potentials and loss-of-function (LOF) K$_{r}$11.1 variants cause Long QT Syndrome which predisposes individuals to fatal cardiac arrhythmias. About 90% of LOF mutations prevent K$_{r}$11.1 intracellular transport (trafficking) to the plasma membrane and prolonged incubation with drugs can sometimes increase K$_{r}$11.1 trafficking and restore K$_{r}$11.1 current (I$_{Kr}$).

Objective: Develop an optimized thallium (Tl$^{+}$)-flux assay to screen a library of clinically approved drugs for increased trafficking of two K$_{r}$11.1 potassium channel variants.

Methods: We developed a novel Tl$^{+}$-based fluorescent assay and HEK-293 cells expressing K$_{r}$11.1 trafficking-deficient variants (K$_{r}$11.1-G601S-G965*X and K$_{r}$11.1-N470D) to screen 1900 drugs (three replicates each) for increased trafficking. HEK-293 cells were plated on 384-well, clear bottomed plates with 10 μM drug in individual wells 24-hours before experiments. On the day of experiments, drug was washed out, loaded with thallium-sensitive dye, and imaged using a 384-well fluorescent plate reader. Drug hits were detected using the slope of fluorescence in the assay and calculating the median and median absolute deviation (MAD).

Results: The screen detected a total of 80 drugs (average >3 MADs) that increased K$_{r}$11.1 trafficking in both variants. Most drugs that increase K$_{r}$11.1 trafficking inhibit the channel acutely, so we next screened acute and 24-hour drug effects on K$_{r}$11.1-WT channel and eliminated drugs that block the channel. Concentration response curves (1 nM to 25 μM) were generated from 40 drugs that had <20% acute block at 10 μM, a tolerable side effect profile and increased trafficking of K$_{r}$11.1. Seven drugs increase K$_{r}$11.1 trafficking at clinically relevant concentrations.

Conclusion: We discovered clinically available drugs that could be readily tested as treatment for patients with Long QT Syndrome caused by trafficking deficient K$_{r}$11.1 variants.

PO-616-04

A NOVEL CPVT-ASSOCIATED CALMODULIN MUTATION CAUSES SEVERE CA$^{2+}$ LEAKAGE FROM SARCOPLASMIC RETICULUM IN IPSC MODEL BY ACTIVATING THE CARDIAC RYANODINE RECEPTORS

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Background: CPVT is a heritable cardiac arrhythmia syndrome characterized by ventricular arrhythmia, especially in response to exercise or stress. CPVT is caused by mutations in the cardiac ryanodine receptor (RyR2) or the cardiac plasmalemmal calcium channel (CACNA1C). Among the RyR2-linked CPVT cases, several mutations, such as I1757K, have been identified. To examine the mechanism underlying the abnormal calcium handling in the RyR2-I1757K mutant, we pursued in silico and in vitro approaches.

Objective: To investigate the role of Ca$^{2+}$ leakage from the sarcoplasmic reticulum (SR) in the RyR2-I1757K mutant.

Methods: We generated induced pluripotent stem cell (iPSC) cardiomyocytes expressing RyR2-I1757K and RyR2-WT. Ca$^{2+}$ handling was evaluated using confocal microscopy and calcium imaging. SR function was assessed by measuring SR Ca$^{2+}$ content.

Results: Calcium imaging revealed increased Ca$^{2+}$ leakage from the SR in RyR2-I1757K compared to RyR2-WT. This leakage was associated with altered SR Ca$^{2+}$ content, indicating impaired SR function. Furthermore, we observed increased Ca$^{2+}$ release kinetics in RyR2-I1757K, suggesting enhanced SR Ca$^{2+}$ release.

Conclusion: Our findings suggest that the RyR2-I1757K mutation causes abnormal Ca$^{2+}$ handling, particularly increased Ca$^{2+}$ leakage from the SR, which may contribute to the pathophysiology of CPVT. These data provide insights into the mechanisms underlying CPVT and offer potential therapeutic targets.