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 activity in basal regions vs. the apex and how this translates into functional responses, has not been explored.

Methods: Male and female cardiac-specific CAMPER reporter mice that report cAMP binding by changes in FRET were used. Hearts were excised and Langendorff-perfused at 12 weeks. Hearts were perfused with drugs that increase cAMP activity in basal regions vs. the apex (n=5, p<0.05). Conversely, in female hearts NE led to a greater change in cAMP activity in the apex vs. 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.

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_+11.1 (HERG) VARIANTS

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

Objective: Develop an optimized thallium (Tl^+)–flux assay to screen a library of clinically approved drugs for increased trafficking of two K_+11.1 potassium channel variants.

Methods: We developed a novel Tl^+–based fluorescent assay and HEK-293 cells expressing K_+11.1 trafficking-deficient variants (K_+11.1-G601S-G965*X and K_+11.1-N470D) to screen 1900 drugs (three replicates each) for increased K_+11.1 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_+11.1 trafficking in both variants. Most drugs that increase K_+11.1 trafficking inhibit the channel acutely, so we next screened acute and 24-hour drug effects on K_+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_+11.1. Seven drugs increase K_+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_+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: The potassium channel K_+11.1 plays an important role in repolarization of cardiac action potentials and loss-offunction (LOF) K_+11.1 variants cause Long QT Syndrome which predisposes individuals to fatal cardiac arrhythmias. About 90% of LOF mutations prevent K_+11.1 intracellular transport (trafficking) to the plasma membrane and prolonged incubation with drugs can sometimes increase K_+11.1 trafficking and restore K_+11.1 current (I_+K_+)....
Background: Calmodulin (CaM) is an intermediate calcium-binding messenger protein and it is very unique that three genes (CALM1-3) encode identical CaM proteins in human. Recently, mutations in CALM have been reported to be associated with long QT syndrome (LQTS) and catecholaminergic polymorphic ventricular tachycardia (CPVT). CALM-related CPVT cases are rare and the underlying disease-causing mechanisms have not been fully elucidated in human cardiomyocytes.

Objective: We hypothesized that an inspection paradox could influence the perception of PS lifetimes in cardiac fibrillation recordings, leading to a potential overemphasis on the importance of long lifetime PS. We characterise the effect of a potential inspection paradox in 8 systems of human, animal, and computational fibrillation.

Methods: Computational simulations (Aliev-Panfilov (APV) model, 2D & 3D Atrial Fibrillation (AF) models), experimentally acquired optical mapping AF and Ventricular Fibrillation (VF) data, and clinically acquired human AF and VF were studied. Distributions of all PS lifetimes across full epochs of AF, VF, or computational simulations, were compared with distributions formed from lifetimes of PS existing at 10000 simulated commencement timepoints.

Results: In all systems, an inspection led towards oversampling of PS with longer lifetimes. In AF simulations a PS lifetime shift of +111.8% 1.9% (P < 0.001 for observed vs overall), in realistic 2D simulations of AF +692.9% ±57.7% (P < 0.001), in a 3D computation simulation +691.7%, in optically mapped rat AF +374.6% 88.5% (P = 0.052), in human AF mapped with basket catheters +129.2% ±4.1% (P < 0.05), human AF-HD grid catheters 150.8% 9.0% (P < 0.001), in optically mapped rat VF +171.3% ±15.6% (P < 0.001), in human epicardial VF 153.5% ±15.7% (P < 0.001).

Conclusion: This multi-system study of human, animal, computational atrial and ventricular fibrillation is an illustration of the fundamental importance of an inspection paradox as a source of bias in phase mapping.