of 1850 ± 485 nM and concentration of 243 ± 46 μM for the endogenous Ca^{2+} buffer (n = 16). This provides insight into some of the essential properties of the cytoplasmic Ca^{2+} regulatory machinery in the posterior pituitary. This endogenous buffer will shape the spatiotemporal profile Ca^{2+} rises induced by action potentials and regulate the amount of neuropeptide release.

3119-Pos Board B274

Co-Expression of TRIC-A and Cardiac Ryanodine Receptor affects Store-Overload Induced Calcium Release in HEK293 Cells

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TRIC represents a novel class of trimeric intracellular cation channels located at the sarcoplasmic reticulum (SR) or endoplasmic reticulum (ER) of multiple cell types. These channels regulate the permeability of K ions across the ER/SR and consequently the movement of Ca ions during excitation-contraction coupling. Previously we showed that genetic ablation of TRIC-A lead to compromised K-permeability and Ca release across the SR/ER membrane, supporting the hypothesis that TRIC could function as counter-ion channels that provide the flow of K ions into the SR during the acute phase of Ca release. In the absence of TRIC, overload of Ca inside the ER/SR causes instability of Ca storage and release, leading to stress-induced dysfunction of skeletal and cardiac muscles. Cardiomyocytes isolated from the wild type mice often show spontaneous Ca release during SR Ca overload, also called store-overload induced Ca release (SOICR) that is mediated by the type 2 ryanodine receptor (RyR2). The appearance of SOICR was significantly less frequent in the TRIC-A knockout cardiomyocytes. Biochemical studies revealed that the carboxyl-tail domain of TRIC-A could interact with the RyR channel, supporting the possibility that TRIC-A may directly regulate the Ca release activity. It has been shown that SOICR can be reconstituted in HEK293 cells expressing RyR2. We found that expression of TRIC-A in these RyR2-expressing HEK293 cells could prevent overload of Ca inside the ER and abolish SOICR generation. Together, our data suggest that functional interaction between TRIC-A and RyR can modulate the Ca release process from internal stores.

3120-Pos Board B275

Temperature Dependence of Ip_3-Mediated Signals

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Cytosolic Ca^{2+} is a universal intracellular messenger with a central role in a diverse array of physiological processes. Ca^{2+} signals can emanate from the endoplasmic reticulum (ER), through inositol trisphosphate receptor/channels (IP_3R), as either highly localized signals arising from the opening of one channel (blip) or several clustered channels (puff) or as global Ca^{2+} waves capable of engulfing the whole cell. We previously examined the temperature dependence of global Ca^{2+}signals in *Xenopus* oocytes, finding that upon cooling the frequency of repetitive Ca^{2+} waves slowed while their amplitudes increased markedly. Since then, advances in imaging technology have made it possible to resolve cytosolic Ca^{2+} signals down to the single channel level. Here, we utilized total internal reflection fluorescence (TIRF) microscopy for high-resolution Ca^{2+} imaging in mammalian SH-SY5Y neuroblastoma cells from 12-40°C, in conjunction with cytosolic loading of the slow Ca^{2+} buffer EGTA to inhibit cluster-cluster interactions to record local blips and puffs, or omitting EGTA to record global Ca^{2+} waves. We found that the amplitudes and spatial spread of blips and puffs showed little temperature dependence, whereas their kinetics (durations and latencies) were markedly accelerated by increasing temperature. In contrast, the amplitudes of Ca^{2+} waves increased appreciably at lower temperatures, probably resulting from longer durations of IP_3R channel openings. Several parameters, including puff and blip durations, puff latency and frequency, and frequency of repetitive Ca^{2+} waves showed a biphasic temperature dependence on Arrhenius plots. In all cases the transition temperature occurred at about 25°C, possibly reflecting a phase transition in the lipids of the ER. As IP_3-evoked Ca^{2+} signals were qualitatively similar at 25°C and 36°C, room temperature experiments should reflect physiological responses at body temperature, but cooling to 12°C, or lower, may be advantageous to improving optical resolution of channel gating kinetics.

3121-Pos Board B276

Muscle-Specific Microrna miR-208 Regulates Calcium Handling Release in Cardiomyocytes by Targeting Phosphodiesterase 4D and Calcineurin

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MicroRNAs are small non-coding RNAs which act as endogenous regulators of gene expression, have gained much attention in the past several years due to increasing evidence of their involvement in numerous pathological processes including cardiac disease. Several microRNAs have been implicated in heart failure (HF) and sudden cardiac death (SCD) via regulation of calcium handling in cardiac myocytes. Upregulation of muscle-specific miRNA miR-208 has been associated with adverse clinical outcomes including acute failure and SCD in patients with dilated cardiomyopathy, while therapeutic inhibition of miR-208 was demonstrated to improve survival and cardiac function in the rat model of HF. However, the molecular mechanisms underlying such functional effects of miR-208 dysregulation remain largely unexplored. We hypothesize that miR-208 is an important regulator of calcium handling by targeting components of phosphorylation-dephosphorylation system in cardiac myocytes. An experimental model of miR-208 overexpression in rat ventricular myocytes was generated via adenoviral infection. Its effects on Ca^{2+} handling and pro-arrhythmic activity was investigated using confocal microscopy for Ca^{2+} imaging, western blot analysis, and luciferase reporter assays. Under conditions of beta-adrenergic stimulation we observed decreased Ca^{2+} transient amplitude, reduced SR Ca^{2+} content, faster onset of pro-arrhythmic spontaneous Ca^{2+} waves, and increased spark frequency in miR-208 overexpressing cells. Additionally, there was increased phosphorylation of a negative regulator of SERCA, phospholamban, and ryanodine receptors as well as decreased levels of putative targets phosphodiesterase 4D and calcineurin in miR-208 overexpressed myocytes. We can conclude that miR-208 affects Ca^{2+} handling by regulating the expression of proteins involved in phosphorylation-dephosphorylation of ryanodine receptors and phospholamban. This mechanism may contribute to the increased risk of arrhythmia and SCD in cardiac disease states accompanied by miR-208 upregulation including HF.

3122-Pos Board B277

Ros in Cardiac Calcium Signaling

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Reactive oxygen species (ROS) are thought to play an important role in the pathophysiology of diverse cells including those in the heart. In addition, recent work by many investigators suggests that there is a significant dynamic signaling role of ROS in many cell types, including cardiomyocytes. Using isolated single heart cells from rat and mouse, confocal imaging and cellular electrophysiology, we investigated the roles of transient elevations of ROS by using a rapid superfusion system to exogenously apply H_2O_2 (100 [[Unsupported Character - Symbol Font μ]]M). We examined Ca^{2+} sparks, $[Ca^{2+}]_i$ transients and membrane currents. We also investigated the roles of the secondary messengers PKA and CaMKII in the ROS-dependent signaling that we examined. Clear alterations of some Ca^{2+} signals were observed with complex contributions from PKA- and CaMKII dependent signals. The roles of mitochondria and NADPH oxidases in this process are discussed.

3123-Pos Board B278

Detection of Calcium Gradients in Live Ex Vivo Human Epidermis and 3D Human Epidermal Equivalents using Phasor Analysis of Two-Photon Excitation Fluorescence Lifetime Imaging (FLIM) and the Genetically Encoded ER Calcium Sensor D1Er

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Ionic gradients regulate the function and the structure of many tissues and organs. However, traditional techniques used to measure ionic concentrations in tissue (such as Proton induced X-ray Emission or ion capture cytochemistry) are often highly invasive (requiring fixation and dehydration of the specimen) and offer poor spatial resolution. In skin, Ca^{2+} gradients have been shown to regulate epidermal barrier function, homeostasis and repair after perturbation, but a clear understanding of the mechanisms regulating such gradients is still lacking. We applied the phasor analysis approach to two-photon excitation FLIM measurements of Calcium Green 5N to quantify and localize Ca^{2+} pools in human epidermis before and after barrier perturbation. The high spatial resolution offered by this method and the data analysis performed allowed us to measure intracellular average calcium concentrations directly in tissue and detect a previously unknown heterogeneous distribution of Ca^{2+} concentrations in the proliferative epidermal basal layer following barrier disruption. More importantly, this approach revealed that the extracellular space in epidermis is much narrower than previously thought. In fact, most epidermal Ca^{2+} localizes to intracellular stores, not extracellular space. To test the hypothesis that epidermal barrier repair after experimental barrier abrogation is driven by