# Local Calcium Signaling

**3010-Pos Board B115**
**Familial Alzheimer's Disease Mutations in Presenilin-1 and Store-Operated Calcium Entry**
**Maria Ryazantseva**, Lyubov Glushankova, Ilya Pozdnyakov, Ilya Bezprozvanny, Elena Kaznacheyeva.
Alzheimer's disease (AD) is a progressive and irreversible neurodegenerative disorder. Familial AD (FAD) mutations in presenilins have been linked to $Ca^{2+}$ signaling abnormalities. Presenilins (PS) are 50 kDa proteins in the endoplasmic reticulum (ER) membrane. The cleaved presenilins are well known as catalytic components of a gamma-secretase, which cleaves the amyloid precursor protein (APP) and releases the amyloid beta-peptide. In addition uncleaved presenilins function as passive ER $Ca^{2+}$ leak channels which control steady-state ER $Ca^{2+}$ levels. It was found that many FAD mutations in presenilins result in loss of ER $Ca^{2+}$ leak function, leading to ER $Ca^{2+}$ overload and supra-normal Ca2+ release from the ER. The ER $Ca^{2+}$ leak function of presenilins is independent of their gamma-secretase activity. We suggested that presenilins affect store-operated calcium influx (SOC) by controlling the filling state of ER $Ca^{2+}$ stores. To determinate the influence of FAD presenilins mutations on SOC we performed a series of patch-clamp experiments in whole-cell mode. PS1-M146V and PS1-ΔE9 mutants have been shown to have loss and gain of ER $Ca^{2+}$ leak channel function respectively. A decrease in maximum amplitude and speed of SOC current activation was observed in SK-N-SH neuroblastoma cells and primary culture of rat hippocampal neurons transfected with PS1-M146V mutant comparing to wild type PS1 transfected cells. An increase in maximum amplitude and speed of SOC current activation was observed in cells transfected by PS1-ΔE9 mutant comparing to wild type PS1. In experiments with triple transgenic AD mice hippocampal neurons (3XTg mice; KI-PS1M146V, Thy1-APPKM670/671NL, Thy1-tauP301L) the maximum amplitude of SOC were decreased comparing to WT, but the speed of activation was the same for 3XTg and WT hippocampal neurons. Electrophysiological properties of all impaired SOCs suggest that TRPC1 is the main target of presenilins FAD mutations affect.

**3011-Pos Board B116**
**Application of Designed Calcium Sensors with Fast Kinetic Responses**
**You Zhuo**, Shen Tang, Yusheng Jiang, Chen Zhang, Florence Reddish, Jenny Jie Yang.
Transient change of cytosolic calcium level leads to physiological actions, which are modulated by the intracellular calcium store, as well as membrane calcium channels. To probe fast calcium responses in high calcium environments, there is a pressing need to develop calcium sensors to overcome the limitation of relatively slow kinetics of current GECIs with τ value around several hundred milliseconds. We have developed single green fluorescence protein-based calcium sensors, with tunable calcium binding affinity and fast kinetics. In this study, we first report our further development of a red calcium sensor using our design strategy. We then report our applications of the developed calcium sensors to monitor endoplasmic reticulum (ER) calcium release in several cell lines responding to perturbations of extracellular calcium signaling. The effects of various drugs as channel and pump inhibitors and activators have also been examined using our developed calcium sensors targeted to calcium channels in the ER membrane.

**3012-Pos Board B117**
**Rational Design and Structural Analysis of Calcium Biosensors and their Application to the Study of SR/ER Calcium Dynamics**
**Shen Tang**, You Zhuo, Yusheng Jiang, Hing-Chueng Wong, Zhong-min Wang, Osvaldo Delbono, Jenny J. Yang.
Quantitative, real-time detection of Ca2+ fluctuations in intracellular organelles is essential to determining the mechanism of Ca2+-dependent signaling. Here, we developed genetically encoded Ca2+ indicators by creating a Ca2+-binding site in the enhanced green fluorescent protein (EGFP). These novel biosensors allow real-time measurement without perturbing the cells' Ca2+ signaling. Upon binding to Ca2+,these biosensors exhibit single-wavelength fluorescence enhancement and Kd values from 0.1 mM to 1 mM, which are optimal for detecting signaling in the skeletal muscle SR. Excessive biological metal ions, such as K+ or Na+, do not alter their ability to sense Ca2+. In particular, our CaratER can respond to various agonists and antagonists in C2C12, HeLa, and HEK-293 cells. It was expressed in the SR of FDB fibers and successfully monitored Ca2+ signaling under voltage-clamp and application of SR agonists. Its fluorescence decrease in response to sarcolemmal depolarization indicates its fast Ca2+ dissociation rate. These results match our stopped-flow kinetic analysis showing that 40-50% of the fluorescence change finished within the 2.2 ms deadtime, when EGTA rapidly mixed with Ca2+- saturated CaratER. We investigated our sensors' optical and conformational properties using various spectroscopic methods, including high-resonance resolution NMR, and designed red fluorescent protein-based Ca2+ biosensors, which exhibit metal selectivity and large fluorescent changes in response to Ca2+. The pH stability was dramatically enhanced with apparent pKa below 5, so they can monitor Ca2+ signaling in deep tissues and small animals and detect simultaneous Ca2+ changes in various subcellular compartments with multiple colors. In conclusion, our molecular design method achieved biosensors that can reliably monitor Ca2+ signaling in high [Ca2+] environments.

**3013-Pos Board B118**
**Evoked Centripetal $Ca^{2+}$ Activation in Cardiac Purkinje Cells: CICR or $Ca^{2+}$ Diffusion?**
Kazi T. Haq, Rebecca Daniels, Sharene Bungay, **Bruno D. Stuyvers**.
In large mammals, cardiac Purkinje cells (**Pcells**) lack transverse tubules (**T-tubules**). However, Pcells respond to stimulations by massive Ca-releases from the sarcoplasmic reticulum (**SR**) similar to those of ventricular myocytes (**VMs**) with T-tubules. Ca-imaging revealed a wave of elevated Ca-concentration (**Cai**) propagating from the sarcolemma (**SL**) toward the centre of Pcells upon stimulation. Ca-diffusion was proposed to explain this centripetal propagation. A thin layer of SR expressing RyR3 was found ~5μm below the SL while other SR-regions expressed RyR2. *Ca-Induced Ca-Release* (**CICR**) from RyR3-SR-region could relay Ca-signal from Ca-entry to RyR2-Ca-release sites in the core, playing equivalent role as T-tubules in VMs. Our **objective** wasto examine whether centripetal Ca-signaling results exclusively from Ca-diffusion or involved intermediate RyR3-CICR. **Methods:**Pcells were prepared from Yucatan swine (30-40kg) and incubated with Fluo4-AM. Ca-dynamics was assessed by 2D-confocal microscopy (30-100fps); pH7.3,35oC. 26 cells were field stimulated at various external Ca-concentrations (**Cao**). Ca-data were compared with those from a model of Ca-propagation across 3 virtual adjacent SR-Ca-release regions. **Results**: In stimulated Pcells, Ca-increase first happened under SL with amplitude increasing from 0.1 to 0.5μmol/L with Cao from 1 to 4mmol/L. A Ca-wave then propagated uniformly from the SL toward cell center at 100-250μm/s. An overall Ca-transient appeared when Ca-wave reached the core region. When the model included intermediate CICR-region, predicted data were consistent with Pcells observations: minimal amplitude (**minAmp**) of peripheral Ca-increase mediating a central Ca-transient similar to that of Pcells, was 130nmol/L; this generated Ca-propagation with velocity (**propVel**) of 160μm/s; propVel was independent on Cao. In contrast, minAmp and propVel were 220nmol/L and 40μm/s respectively, and propVel increased with Cao when only diffusion was considered. **Conclusion**: Our data were consistent with an intermediate CICR-mechanism which ''boosts'' Ca-propagation between SL and core of Pcells.

**3014-Pos Board B119**
**Local Control of Cardiac Sodium-Calcium Exchanger by PMCA in Submembrane Microdomain in Mouse Heart Cells**
**Takao Shioya**.
OBJECT: This study aimed at unveiling functional interaction between the Na/Ca exchanger (NCX) and the plasma membrane $Ca^{2+}$-ATPase (PMCA) on the sarcolemmal membrane of heart cells. METHOD: The Na/Ca exchange current ($I_{NCX}$) was recorded from whole-cell clamped mouse ventricular myocytes under physiological conditions at 37°C. Functions of the ryanodine receptor and SERCA $Ca^{2+}$-pump on the sarcoplasmic reticulum were abolished by using ryanodine and thapsigargin. The $I_{NCX}$ was isolated as $Ni^{2+}$-sensitive current component, under the conditions that eliminate other major membrane current systems. RESULTS: With the $[Ca^{2+}]_i$ strongly buffered with 10 mM-BAPTA, the $I_{NCX}$ was recorded as a time-independent current. However, with the $[Ca^{2+}]_i$ only weakly buffered with 0.1 mM-BAPTA, the $I_{NCX}$ showed a small current amplitude and a slow activation time-course. This was not observed when the $[Ca^{2+}]_i$ was strongly buffered (with 10 mM-BAPTA). Inhibition of PMCA by intracellular administration of orthovanadate ($VO_4^{3-}$) dramatically increased the amplitude of $I_{NCX}$ and accelerated its activation kinetics. At the same time, orthovanadate shifted the $[Ca^{2+}]_i$-dependence of the $I_{NCX}$ amplitude ($EC_{50}$ = 0.97 μM) to lower levels ($EC_{50}$ = 0.40 μM). Moreover, similar effects were observed by intracellular application of a selective PMCA inhibitor 5(6)-carboxyeosin. CONCLUSION: The PMCA regulates the operation of the NCX by altering local $[Ca^{2+}]_i$ level around the NCX molecule. Because PMCA is driven by ATP, this functional coupling might serve as a mechanism that the intracellular metabolic status to the $[Ca^{2+}]_i$ regulation and $Ca^{2+}$ signaling in heart cells.