and likely plays a key regulatory role in the spatial temporal pattern of Ca^{2+} signaling underlying synaptic plasticity.

2595-Pos Board B365

Modulating Drug Effects of Metabotropic Glutamate Receptor 1 Alpha (mGluR1α) by Extracellular Ca^{2+}

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Metabotropic glutamate receptor 1α (mGluR1α), known as a member of the family C GPCRs, couples to Gq and modulates consequent PLC activity, IP3 accumulation and intracellular Ca2+ extrusion from ER lumen. The mGluR1α is abundantly expressed in central nervous system and has been shown to be responsive to the slow phase of the action potential in post-synapses, and to be involved in chronic neuronal degenerative diseases, like Parkinson's disease, Huntington's disease and Alzheimer's disease. We have predicted a potential Ca2+ binding site adjacent to the binding site to reported endogenous agonist glutamate and antagonists. In this study, we have applied single cell imaging, IP1 binding, and radioactive assay to probe the effect of extracellular calcium in modulating various types of the drugs of modulating mGluR1a such as agonists, antagonists and allosteric modulators. We have shown that extracellular Ca2+ enhances the agonist's activation of intracellular calcium responses of mGluR1α by increasing the drug binding to the receptor. In addition, extracellular Ca2+ also differentially modulates the inhibition of the receptor by antagonists and allosteric modulators. Our studies open a new avenue for modulating drug effects and developing novel drugs against neurodegenerative diseases.

2596-Pos Board B366

Depolarization-Induced Intracellular Ca^{2+} Release in Postganglionic Sympathetic Neurons from Adult Mice

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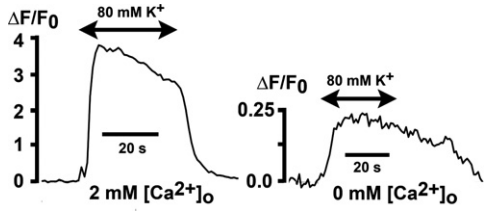
Ca^{2+} influx through voltage-activated plasmalemmal Ca^{2+} channels provides a trigger for Ca^{2+} release from internal Ca^{2+} stores in mammalian postganglionic sympathetic neurons. Here we examined whether a Ca^{2+} influx-independent, depolarization-induced component contributes to the rise in intracellular Ca^{2+}. Exposure of postganglionic sympathetic neurons isolated from adult mice to a high K^+ (80 mM), normal Ca^{2+} (2 mM) solution for 30 s caused sustained membrane depolarizations from -58.9 ± 3.4 mV to -12.8 ± 0.8 mV (mean ± SEM; 38 cells) and increases in fluo-4 $\Delta F/F_0$ (F indicates fluorescence intensity, and F_0 indicates F at baseline), which rapidly resolved upon repolarization (left Figure; peak $\Delta F/F_0$ averaged 5.20 ± 0.38). Superfusion with a high K^+, Ca^{2+}-free solution caused depolarizations of similar magnitude and small-amplitude increases in $\Delta F/F_0$ (mean peak $\Delta F/F_0 = 0.41 \pm 0.03$; 29 cells) with slow decay upon repolarization (right Figure). Thapsigargin (1 μM) or the IP_3 receptor inhibitor 2-APB (20 μM), but not ryanodine (20 μM) or nifedipine (50 μM), abrogated Ca^{2+} rises evoked by high K^+ in the absence of external Ca^{2+} without affecting the membrane voltage response. Thus, sympathetic neurons exhibit depolarization-induced Ca^{2+} release from IP_3-sensitive stores, linking electrical activity and a rise in cytoplasmic Ca^{2+}.



2597-Pos Board B367

CaMKII Regulation of Calcium Homeostasis and Neuronal Activity

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Calcium/calmodulin-dependent kinase II (CaMKII) is a multifunctional serine/threonine protein kinase that regulates multiple ion channels and receptors that are essential for neuronal activity and plasticity. To explore the role of CaMKII in regulating neuronal calcium homeostasis, we applied pharmacological inhibitors of CaMKII (10 micromolar tat-CN21, tat-AIP, and myr-AIP) to cultured cortical and hippocampal neurons and measured changes in intracellular calcium levels using Fluo-4AM and Fura-2FF. CaMKII inhibition induced a rapid increase in intracellular calcium levels. Dysregulated calcium signaling was not limited to neurons, as CaMKII inhibitors also initiated calcium waves in cultured astrocytes that preceeded neuronal effects. Inhibitors that disrupt neuronal activity, VGSCs, and AMPA-Rs, block this calcium dysregulation in neurons. Although L-type calcium channel blockers had no effect, eliminating extracellular calcium, inhibiting NMDA-Rs or N-type calcium channels did prevent calcium dysregulation in neurons, implicating aberrant glutamate release and/or post-synaptic activity in this calcium dysregulation. Increased glutamate levels (2-4 micromolar) were observed in the media following CaMKII inhibition. Enzymatic buffering of glutamate in the media prevented the aberrant calcium influx. Finally, to directly test whether CaMKII inhibition increases neuronal activity, voltage-clamp electrophysiology was utilized to measure action potential generation to a depolarizing ramp current. Unlike inactive controls, CaMKII inhibitors induced a three-fold increase in the number of action potentials when applied in the patch pipette. In total, a loss of CaMKII activity results in dysregulation of intracellular calcium homeostasis and a subsequent dysregulation of glutamate signaling and neuronal excitability. Thus, CaMKII not only responds to neuronal activity via changes in intracellular calcium levels, it also appears to serve as a master regulator of neuronal excitability through regulation of calcium homeostasis.

2598-Pos Board B368

Ca^{2+} Signaling in Rat Ventricular Myocytes

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We employ a coupled electromechanical mathematical model to better understand the biophysical basis of the force-frequency response (FFR) in rat ventricular myocytes under voltage clamp conditions. The model extends our previous work on calcium signaling in the cardiac dyad, and the regulation of Ca^{2+}-concentration in the myoplasm. The present work is focused on achieving a better understanding of mechanisms involved in the rat FFR. Specifically, we examine the role of calmodulin (CaM) in modulating the key control variables Ca^{2+}/calmodulin-dependent protein kinase-II (CaMKII), calcineurin (CaN), and cyclic adenosine monophosphate (cAMP), as they mediate a rate-dependent effect on various intracellular targets controlling the FFR.

Our electrochemical model consists of an electrical-equivalent model for the cell membrane; dyadic, myoplasmic and sarcoplasmic reticulum (SR) fluid-compartments; and a modified model of the contractile system by Rice et al. We incorporate frequency-dependent CaM-mediated and spatially heterogenous interaction of the proteins CaMKII and CaN with their principal targets (dihydropyridine (DHPR) and ryanodine (RyR) receptors, and the SERCA pump). Also included are the rate-dependent effects of phospholamban (PLB) on SERCA pump; cAMP on the DHP-sensitive Ca^{2+} channel; and the enhancement in SERCA pump activity via phosphorylation of PLB.

Investigators using multicellular rat ventricular preparations have recorded both positive and negative peak FFRs. Under specific conditions, our VC model can generate either a positive or a negative FFR, while providing mechanistic understanding of its genesis. In addition, the model provides quantitative insight into rate-dependence of CICR by investigating the frequency-dependence of each contributing factor. Since several aforementioned multicellular studies were conducted at different temperatures, we also investigated the temperature-dependence of FFR.

Our modeling study suggests that cAMP-mediated stimulation and rate-dependent CaMKII-mediated up-regulation of the trigger current $I_{Ca,L}$ are key mechanisms underlying the inconsistency in FFR observations in multicellular rat ventricular tissue.

2599-Pos Board B369

Inhibition of Akt Attenuates Isoproterenol-Induced RyR-Dependent Diastolic Ca2+ Release in Rabbit Ventricular Myocytes

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Cardiac ryanodine receptor (RyR)-dependent diastolic SR Ca2+ release is increased by beta-adrenergic receptor (ß-AR) stimulation. Increased diastolic RyR activity can lead to arrhythmogenic spontaneous SR Ca2+ release. Our studies have shown that calmodulin-dependent protein kinase II (CaMKII) activation by nitric oxide (NO) implicates nitric oxide synthase (NOS) in the pathway. Here we investigate the role Akt may play in activating NOS, thus increasing diastolic SR Ca2+ release during ß-AR stimulation in isolated rabbit ventricular myocytes. Western blot analysis showed a dose-dependent increase in phosphorylated Akt in response to the β-AR agonist isoproterenol (ISO). Cytosolic Ca2+ was measured using fluo-4 loaded intact myocytes. SR Ca2+ was varied by field-stimulated to steady state at different frequencies. Tetracaine