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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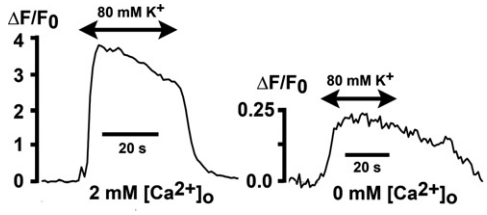
Ca^{2+} influx through voltage-activated plasmalemmal Ca^{2+} channels provides a trigger for Ca^{2+} release from internal Ca^{2+} stores in mammalian postganglionic sympathetic neurons. Here we examined whether a Ca^{2+} influx-independent, depolarization-induced component contributes to the rise in 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

(1mM) was used to rapidly and reversibly block the RyR. The tetracaine-dependent shift of Ca2+ from the cytosol to the SR is proportional to diastolic Ca2+ release. Primary myocytes were treated with 250nM ISO with and without Akt inhibitor-X (AIX; 5μM; 30min pre-incubation and continuous perfusion). ISO-induced increase in the diastolic SR Ca2+ release was abolished by the treatment of myocytes with AIX. When data were selected such that SR[Ca2+] was matched in each group (ISO: 155.22 ± 5.1μM; ISO+AIX: 153.92 ± 3.8μM), myocytes treated with ISO had significantly higher tetracaine-dependent increase in diastolic SR[Ca2+] (12.43 ± 3.8μM) vs. those treated with ISO and AIX (1 ± 2.2μM) (P=0.01, t-test). The results suggest that the ISO-dependent increase in diastolic SR Ca2+ release is dependent upon Akt. To further test this hypothesis myocytes were cultured for 24 hours with adenovirus to express a dominant-negative Akt construct (Akt-dn). Preliminary evidence shows reduced diastolic SR[Ca2+] release for a given SR[Ca2+] in Akt-dn myocytes. This evidence indicates that Akt activation may be an important upstream effector involved in the β-AR-induced diastolic SR Ca2+ release.

2600-Pos Board B370

Ranolazine Alters the Properties of Localized Ca^{2+} Sparks and Inhibits Global Ca^{2+} Waves in Rat Myocytes

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The effect of ranolazine (10 μM) on local Ca^{2+} regulation was studied in isolated rat ventricular myocytes. Cells were loaded with fluo-4 AM and Ca^{2+} was detected using line-scan confocal imaging. Ranolazine increased Ca^{2+} spark frequency by 57.8 ± 17% ($p<0.05$, n=29). This was accompanied by significant ($p<0.05$) decreases in Ca^{2+} spark amplitude (44.5 ± 0.1%), duration (16.5 ± 1.9%) and width (5.6 ± 1.7%) resulting in a decrease in the calculated 'spark mass' of 41.3 ± 5.4% (data from 15-29 cells). In cells initially paced at 0.2 Hz in the presence of forskolin (10 μM), cessation of stimulation was followed by spontaneous diastolic Ca^{2+} waves. After introduction of ranolazine (but not in its absence), the frequency of spontaneous Ca^{2+} waves decreased markedly over 10-15 minutes and waves were completely abolished in 5 of 11 cells. Inhibition of Ca^{2+} waves also occurred in permeabilized cells, suggesting an intracellular site of action. We propose that the ranolazine-induced decrease in Ca^{2+} spark mass may contribute to inhibition of pro-arrhythmic Ca^{2+} waves by reducing the probability of saltatory propagation between Ca^{2+} release sites.

2601-Pos Board B371

Voltage Dependence of Calcium Spike Characteristics in Rat Cardiac Myocytes

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Integral calcium release fluxes in mammalian cardiac myocytes increase in amplitude not only due to the increased recruitment of individual release sites but also due to their increased synchronization (1). Here we characterize the voltage dependence of synchronization of individual release sites at high temporal resolution. Calcium spikes and calcium currents elicited in isolated rat ventricular myocytes (2) by 80 ms voltage pulses from −50 to −40 - +50 mV were recorded using Leica TCS AOBS confocal microscope (2 kHz line scan mode) and Axopatch 200B patch-clamp amplifier, respectively. Calcium spikes were identified and analyzed by a new Matlab-based software that highly increased the productivity and reliability of approximation of calcium spikes by the kinetic model of local calcium release (2). Calcium spikes were described by their amplitude, onset latency, time-to-peak, FDHM, time constants of activation and termination, and fractional probability of activation. The synchrony of calcium spikes increased with depolarization and could be fully explained by the voltage dependence of their onset latency that reached a lower limit of about 4 ms at depolarizations between 0 and 40 mV. The rate of calcium spike activation after its onset was independent of the applied voltage or of calcium current amplitude, while the probability of calcium spike activation was related to the voltage dependence of the integral of calcium current. We conclude that both the synchrony of local calcium release activation and recruitment of individual release sites are fully controlled by the activation probability of DHPR calcium channels and by the DHPR-RYR coupling fidelity.

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2602-Pos Board B372

Refractoriness of Calcium Release Units in Rat Cardiac Myocytes

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Calcium release units (CRUs) in cardiac myocytes contain a large number of ryanodine receptors (RyRs), but it seems that only a small fraction of RyRs is being activated during a single calcium release event. Repetitive activity of CRUs is not well understood from the point of refractoriness. We used direct measurement of Ca-spikes activated by calcium current (ICa) to unveil the refractoriness of CRU firing from the properties and occurrence of calcium spikes under control conditions and in the presence of the DHPR activator FPL.

ICa was activated by voltage pulses from 50 to 0 mV under whole-cell patch-clamp. Ca-spikes were measured by confocal microscopy using Ca2+ indicators (Fluo-3 and Oregon Green BAPTA-5N) in the presence of EGTA. A large majority of CRUs responded to stimulation by a single Ca-spike, while 13.4 % produced two subsequent (twin) Ca-spikes. Early Ca-spikes (single spikes and the first of twin Ca-spikes) had similar latencies, while the second Ca-spikes were significantly delayed. Amplitude distribution of the early Ca-spikes consisted of four quantal levels with equal amplitudes and binomially distributed frequency of occurrence, supporting the hypothesis that different Ca-spike amplitudes are due to a different number of independent quantal levels, likely individual RYR openings. The probability of occurrence of second Ca-spikes was inversely proportional to the quantal size of the early Ca-spike. In the presence of FPL, the probability of occurrence of twin spikes was decreased relative to control, despite persistent activation of DHPR receptors.

We conclude that refractoriness of local calcium release does not result from Ca-dependent DHPR inactivation, but rather it is caused by inactivation of the release unit due to local depletion of the SR.

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2603-Pos Board B373

Cyclopiazonic Acid Differently Modulates Junctional and Non-Junctional Ca^{2+} Transients in Rat Atrial Myocytes

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Atrial myocytes have two functionally distinguished sarcoplasmic reticulums (SRs): those at the periphery close to the cell membrane, and those at the cell center (interior) not associated with the cell membrane. To know the extent to which the peripheral and central Ca^{2+} release sites are controlled by SR Ca^{2+} pump the effects of cyclopiazonic acid (CPA), the inhibitor of SR Ca^{2+} pump, on peripheral junctional Ca^{2+} releases and central non-junctional Ca^{2+} releases were compared. Rapid (230 Hz) 2-D confocal Ca^{2+} imaging was used in field-stimulated rat atrial myocytes. Diastolic Ca^{2+} level was increased by CPA dose-dependently. At lower doses (0.2 and 0.4 μM), the CPA-induced increase in diastolic [Ca^{2+}] was larger in the center. Low dose of CPA increased the systolic Ca^{2+} level and magnitude of Ca^{2+} transients shortly in the cell periphery. However, high concentration (5 μM) of CPA significantly decreased them in the cell periphery. In contrast, low doses of CPA increased central Ca^{2+} transients slowly and the effect was prolonged. High dose of CPA did not reduce central Ca^{2+} transients. Ca^{2+} transient decay was slowed by CPA dose-dependently. The CPA-induced deceleration of Ca^{2+} transient decay was significantly stronger in the center than in the periphery. The velocity of Ca^{2+} propagation wave during action potential was dose-dependently reduced by CPA. This result was consistent with more increase in the time-to-peak of central Ca^{2+} release. Our data suggest that the Ca^{2+} pump may play more important role in non-junctional Ca^{2+} decay in beating atrial myocytes. In addition, our data suggest that active Ca^{2+} uptake via the SR Ca^{2+} pump may facilitate the transverse Ca^{2+} propagation wave in atrial myocytes on depolarization.

2604-Pos Board B374

Eccentric Exercise Elicits Elevation of Resting Calcium in Skeletal Muscle. Periodic Acceleration Enhanced the Recovery

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Eccentric exercise (EC) can cause transient muscle damage, including muscle injury, muscle protein release into the plasma, an acute-phase immune response, and a decrease of muscle performance. Periodic acceleration (pGz) is a novel treatment that stimulates nitric oxide (NO) production by cNOS. Male mice (25-32 g) were divided in 4 groups (5/group). *Group A*: non-exercised (control); *Group B*: EC (45 min downhill exercise); *Group C*: EC followed by 30 min of pGz at 480 cpm daily starting on day 0 for 10 days; *Group D*: EC followed by 30 min of pGz, treated with L-NAME, 4 days prior and