with a maximum insertion pressure of 30 mN/m, which is in the range of the lateral pressure postulated for biological membranes. Moreover, results show that the size and the charge of the polar head group of phospholipids are also implicated in recoverin adsorption in monolayers. Furthermore, polarization-modulated infrared reflection absorption spectroscopy (PM-IRRAS) has been used to determine the secondary structure and orientation of recoverin in monolayers. PM-IRRA spectra indicated the prevalence of α-helices in the secondary structure of recoverin, which is consistent with its known structure. In contrast, non-myristoylated recoverin is quickly denatured after its adsorption in monolayers. Finally, the amide I/amide II ratio allowed to determine that the α-helices of myristoylated recoverin are oriented perpendicular to the plane of the monolayer.

603-Pos Board B482

Characterization Of Ca^{2+} Photo-release From DM-nitrophen Using Photothermal Beam Deflection

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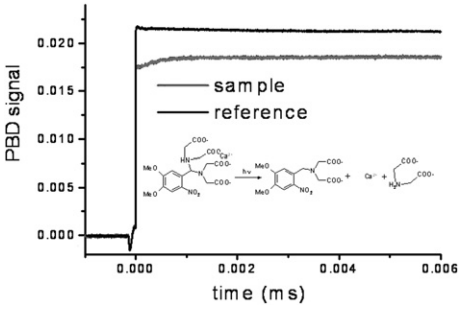
DM-nitrophen is a popular caged calcium compound that allows for a rapid increase in calcium concentration from hundred nanomolar to tens to hundred micromolar level on the microsecond timescale. To fully understand the mechanism of calcium photo-release from DM-nitrophen, we have used photothermal beam deflection technique to investigate time-profiles of volume and enthalpy changes associated with DM-nitrophen photodissociation. Our data show that the photodissociation of calcium loaded DM-nitrophen occurs as a two-step process. The first step takes place within ~ 10 μs upon photolysis and is associated with a volume decrease of -7 mL mol^{-1} and enthalpy change of 66 kcal mol^{-1}. On the longer timescale ($\tau = 200$ μs), the second event with a positive volume change of 7 mL mol^{-1} and enthalpy change of 8 kcal mol^{-1} was detected. These data are in agreement with the previous fluorescence studies showing that calcium release from DM-nitrophen occurs as a two step process.



604-Pos Board B483

Altered Structure Of The Cerebellar Granule Cell Layer Of Mice Lacking Calretinin

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Calcium binding proteins, such as calretinin, are abundantly expressed in distinctive patterns in the central nervous system, but their physiological functions remain poorly understood. Calretinin is highly expressed in cerebellar granule cells and calretinin deficient mice exhibit alterations in motor coordination. Using confocal microscopy, we demonstrate that the cerebellar cortex of calretinin deficient mice exhibit a significantly decreased granule cells density. Interestingly, it has been shown that the migration of granule cells is tightly associated with intracellular calcium oscillations . Therefore, we hypothesize that an alteration of these calcium oscillations in calretinin deficient mice could be involved in the observed morphological alterations. To test this assumption, we are currently developping two strategies. First, using confocal microscopy and cerebellar microexplant culture, we are studying calcium oscillations occuring during granule cell migration in the wild type control and calretinin knock-out mice. This allow us to characterize the impact of variations in calcium buffering capacity over neuronal development and on the generation of the calcium oscillations observed during the granule cell migration. On the other hand, we are developping a theoretical model to study the impact of calcium buffering modifications on the dynamics underlying the observed calcium oscillations . This dedicated computational model will shed light on the possible mechanism responsible for the modulation, by calretinin, of calcium oscillations during the granule cell migration.

605-Pos Board B484

Characterization Of Zebrafish (Danio Rerio) NCX4: A Novel Na/Ca Exchanger With Distinct Electrophysiological Properties

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Members of the Na+-Ca2+ exchanger (NCX) family are important regulators of cytosolic Ca2+ in myriad tissues and are highly conserved across a wide range of species. Three distinct NCX genes and numerous splice variants exist in mammals, many of which have been characterized in a variety of heterologous expression systems. Recently, however, we discovered a fourth NCX gene (NCX4) which is found exclusively in teleost, amphibian and reptilian genomes. The zebrafish (Danio rerio) NCX4a encodes for a protein of 939 amino acids and shows a high degree of identity with known Na+-Ca2+ exchangers. Although knock down of NCX4a activity in zebrafish embryos has been shown to alter left-right patterning, it has not been demonstrated that NCX4a functions as a Na+/Ca2+ exchanger. In this study, we: 1) demonstrate for the first time that this gene encodes for a novel NCX; 2) characterize the tissue distribution of zebrafish NCX4a and 3) evaluate its kinetic and transport properties. While ubiquitously expressed, the highest levels of NCX4a expression occur in the brain and eyes. NCX4a exhibits modest levels of Na+-dependent inactivation and requires much higher levels of regulatory Ca2+ to activate outward exchange currents. NCX4a also exhibited extremely fast recovery from Na+-dependent inactivation of outward currents, faster than any previously characterized wild-type exchanger. While this result suggests that the I1 inactive state of NCX4a is far less stable than in other NCX family members. We have demonstrated that a new putative member of the NCX gene family NCX4a encodes for a Na+/Ca2+ exchanger with unique properties including an extremely rapid recovery from Na+-dependent inactivation. These data will be useful in understanding the role that NCX4a plays in embryological development as well as in the adult where it is expressed ubiquitously.

Calcium Signaling Pathways

606-Pos Board B485

Modeling [Na+] in PM-SR Nanodomains of Vascular Smooth Muscle Cells

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We present a stochastic computational model aimed at elucidating the mechanism of site-specific signalling between a source and a target ionic transporter, both of which are localized on the plasma membrane (PM) and are part of a nanodomain: nanometer-scale subplasmalemmal signalling compartments comprising the PM, the sarcoplasmic reticulum (SR), Ca2+ and Na+ transporters (channels, exchangers and pumps), and the intervening cytosol. In this chain of events, the physical and functional link between non-selective cation channels (NSCC) and Na+/Ca2+ exchangers (NCX) needs to be elucidated in view of two interesting recent findings: the identification of the TRPC6 as the NSCC in VSM cells and the observation of localized Na+ transients following purinergic stimulation of these cells. Having previously helped clarify the signalling step between NCX and SERCA behind sarcoplasmic reticulum (SR) Ca2+ refilling, this quantitative approach now allows us to make inroads into this important signalling step. We have implemented a random walk (RW) Monte Carlo (MC) model with simulations mimicking a Na+ diffusion process originating at the NSCC within PM-SR junctions. Physical features of the system (junctional dimensions, diffusivity of Na+ in cytosol, channel capacity, etc.) were obtained in our laboratory and from the existing literature. The model calculates the average [Na+] in the junction and also produces iso-concentration profiles for [Na+] as a function of distance from the Na+ source. It also analyzes the influence of the junctional geometry on the signaling ability of the nanodomain. Our results emphasize the necessity of a strategic juxtaposition of the relevant signalling channels and organelles to form nanodomains that permit adequate [Na+] build-up to provoke NCX reversal and Ca2+ influx via NCX eventually to refill the SR during asynchronous Ca2+ waves.

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A 3d Pseudo-stochastic Model Of Intercellular Calcium Signaling In Smooth Muscle

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We present a general 3D pseudo-stochastic model of intercellular calcium signaling (ICS) in smooth muscle, composed of independently tunable units aggregated through a flexible convolution procedure. ICS ensures propagation and synchronicity of myocytes' contraction within muscular syncytia from

the walls of hollow organs. Increase in cytosolic [Ca^{2+}] results from agonists released by nerve terminals and binding ionotropic membrane receptors thereby opening Ca^{2+} channels or metabotropic receptors thus activating Ca^{2+}-mobilization through secondary messengers. Extra [Ca^{2+}] in the proximity of ER ryanodine receptors stimulates Ca^{2+}-induced Ca^{2+}-release from ER stores that is inhibited over certain [Ca^{2+}]. The model considers also regenerative ICR propagation through agonist-stimulated-agonist-release by certain hemichannels activated directly by agonists or through activation of certain purinergic receptors. Degradation of bound agonists together with receptor desensitization progressively attenuate both Ca^{2+} influx and Ca^{2+} release from the ER stores. Basal [Ca^{2+}] is then restored by Ca^{2+}extrusion pumps and by resequestering excessive Ca^{2+} within ER stores and mitochondria. The event sequence propagates to neighboring cells by diffusion of agonists through extracellular medium and of Ca^{2+} and second messengers through gap junction channels between coupled cells. Variability of ICS is provided by randomly distributing the myocytes with a given density in a 3D staggered grid and randomly selecting from experimentally determined intervals values characterizing the extracellular compartment, individual cells, cell interconnections and receptor activation and desensitization in healthy and diseased humans and animal models of human diseases. The model allows us to mimic consequences of alterations of individual and combined ICS components to better understand the etiology of abnormal smooth muscular activity and to explore limitless therapeutic avenues of diseases with deleterious effects on the smooth musculature. By determining the statistics of large libraries of simulations, we can evaluate the incidence of various phenotypes.

608-Pos Board B487

Increased Sensitivity to Ischemia in an Early Diabetic Cardiomyopathy: The Role of Calcium Handling

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Diabetic cardiomyopathy is characterized by early-onset diastolic dysfunction and late-onset systolic dysfunction. However, little is known about the mechanisms underlying the response of the diabetic myocardium to ischemia.

Aim - To study the left ventricular (LV) dysfunction and the role of calcium handling in infarcted diabetic mice in an early stage of diabetic cardiomyopathy.

Methods and Results - A cohort of male diabetic *db/db* and age-matched non-diabetic control mice at 10 wk of age was randomly assigned into Sham and myocardial infarction (MI) groups. MI was induced by coronary ligation. Standard echocardiography and tissue Doppler imaging were performed by high-resolution in-vivo imaging system, and diastolic sarcoplasmic reticulum (SR) calcium leak was measured in isolated cardiomyocytes using fluorescence microscope. One month after MI, 75% of the nondiabetic mice survived vs. 55% of the MI diabetic mice ($p=0.04$). A significant LV dilatation was observed in MI diabetic mice compared to nondiabetic ($p=0.03$). Peak systolic tissue velocity (Sm) was 28% lower in MI diabetic mice than in nondiabetic group (non-diabetic: 19 ± 1 vs. 17 ± 2mm/s; diabetic: 18 ± 2 vs. 13 ± 2*mm/s, *$p=0.05$, for Sham and MI, respectively). Peak early diastolic tissue velocity (Em) was decreased in both Sham and MI diabetic mice (17 ± 2* and 16 ± 4* vs. 25 ± 3 and 25 ± 4 mm/s, *$p<0.05$, respectively). Diastolic SR calcium leak was unchanged in 10-wk diabetic mice compared with nondiabetic mice. However, a significant increase diastolic SR calcium leak was observed in MI diabetic mice relative to MI nondiabetic mice.

Conclusion - The altered calcium homeostasis is an important determinant of sensitivity to ischemia and of loss of the ventricular function in the early stage of diabetic heart disease in diabetic mice.

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Calcium Induced Conformational Changes In The Cytoplasmic Tail Of Polycystin-2

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Autosomal dominant polycystic kidney disease (PKD) is the most common, monogenic cause of kidney failure in humans, characterized by the presence of fluid filled cysts in kidneys, liver, pancreas and intestines. Most cases of PKD are linked with mutations in the genes *Pkd1* or *Pkd2*, which encode proteins polycystin-1 (PC1) and polycystin-2 (PC2) respectively. Here we focus on PC2 a calcium (Ca^{2+}) permeable channel in the transient receptor potential (TRP) channel family. PC1 and PC2 interact directly and this interaction is thought to be mediated by their cytoplasmic carboxyl terminal tails. The deletion of the carboxyl terminus of either PC1 or PC2 alters Ca^{2+} signaling; the most common pathogenic mutations in PC2 are premature truncations. We have previously identified two stable domains within the C-terminus of PC2. The first is an EF-hand Ca^{2+} binding domain. The second is a previously unreported coiled-coil domain which we show is responsible for oligomerization of PC2 using Small-Angle X-ray Scattering (SAXS), Analytical Ultracentrifugation and Size Exclusion Chromatography. We show by Isothermal Titation Calorimetry that the EF-hand domain binds Ca^{2+} and that mutations in the predicted Ca^{2+} binding loop abolish the affinity for Ca^{2+}. We hypothesize that the EF-hand domain serves as a Ca^{2+} sensor/switch, and show that PC2 undergoes Ca^{2+} induced conformational changes by NMR, CD, and SAXS. We have completed NMR experiments necessary for high resolution structure determination in the presence and absence of Ca^{2+} and have identified PC2 residues with significant chemical shift changes upon Ca^{2+} titration. We have obtained crystals of the coiled coil domain of PC2 and are optimizing crystals for X-ray diffraction. Structure determination of these cytoplasmic domains will enable a structure-guided analysis of PC2 mediated Ca^{2+} signaling and further investigations into the molecular basis of PKD progression.

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L163,255, a Synthetic Growth Hormone Secretagogue, Raises [Ca^{2+}]$_i$ by Promoting Intracellular Calcium Stores Depletion in Intact Fast-Twitch Fibers of Rat Skeletal Muscle

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The synthetic growth hormone secretagogues, GHS, and the endogenous ghrelin are small molecules proposed as pharmacological tools for the treatment of GH deficiency conditions in view of their ability to stimulate the GH release. Other than in pituitary gland, GHS receptor binding sites are documented in peripheral tissues accounting for a series of GHS pleiotropic activities. Accordingly, a direct action of GHS on skeletal muscle has been proposed, as they reduced resting chloride and potassium conductances in muscle fibres, probably through the activation of a GHS-receptor linked to PLC/PKC/Ca^{2+} signalling (Pierno et al. 2003. *Br. J. Pharmacol*. 139:575–584). By using fura-2 fluorescent measurements, we evaluated the effect of L163,255, a non peptidic GHS, on calcium homeostasis of rat *Extensor Digitorum Longus* (EDL) fibers mechanically isolated. *In vitro* application of L163,255 increased cytosolic calcium concentration, [Ca^{2+}]$_i$, in a dose-dependent manner with an IC_{50} of ~ 300 μM. Particularly, application of 200μM L163,255 led to [Ca^{2+}]$_i$ increase from 26 ± 2 nM to 164 ± 34 nM after 10 min of incubation.Removal of external Ca^{2+} in the bath solution did not abolish L163,255 effects. On the contrary, pre-incubation with the Ca^{2+}-ATPase inhibitor thapsigargin or the mitochondrial permability transition pore inhibitor Cyclosporin A partially and strongly reduced L163,255-induced Ca^{2+} transient respectively, suggesting the involvement of thapsigargin-sensitive calcium stores and mitochondria in the drug action. These data support the presence of GHS receptors on skeletal muscle and highlight that GHS, proposed as therapeutic drugs, by altering calcium homeostasis could interfere with skeletal muscle functionality.

611-Pos Board B490

Multiple Sources of Light-Evoked Intracellular Calcium Increases in *Hermissenda* Type B Photoreceptors

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Previous research suggests that learning-produced changes in excitability and K^+ currents of *Hermissenda* Type B photoreceptors are Ca^{2+}-dependent phenomena. Little information is available concerning the sources and dynamics of Ca^{2+} in these cells. We have used Fura-2 dual-wavelength (340/380 nm) photometry to measure somatic [Ca^{2+}]$_i$ in B cells. Thirty sec light steps (LS) produce a large increase in [Ca^{2+}]$_i$ (~ 246%). To determine the contribution of Ca^{2+}-influx vs Ca^{2+}-release, we measured [Ca^{2+}]$_i$ throughout 5 consecutive LSs in either normal or Ca^{2+}-free ASW (0 mM Ca^{2+}, 30 mM EGTA). Cells exposed to Ca^{2+}-free ASW had a basal [Ca^{2+}]$_i$ much lower than when external Ca^{2+} was present, often below detection limits. Ca^{2+}-free ASW abolished light-induced [Ca^{2+}]$_i$ increases in all 7 cells tested. We next explored the role of voltage-gated Ca^{2+} channels (VGCCs) to Ca^{2+} influx with the use of cobalt (5mM), a VGCC blocker in B cells. Co^{2+} did not affect either basal [Ca^{2+}]$_i$ or light-induced [Ca^{2+}]$_i$ increases ($n = 5$). To assess the contributions of the ER to light-induced [Ca^{2+}]$_i$ changes, B cells were incubated in the ryanodine receptor (RyR) blocker dantrolene (50 μM). Dantrolene reduced the [Ca^{2+}]$_i$ response by ~33% ($n = 5$), and also produced a progressive reduction in basal [Ca^{2+}]$_i$ (~ 60%). Exposure of Ca^{2+}-free ASW cells ($n = 3$) to thapsigargin (TH; 100μM - 1mM, a blocker of the ER Ca^{2+}-ATPase pump) increased basal [Ca^{2+}]$_i$, consistent with store depletion. Collectively, our results indicate that [Ca^{2+}]$_o$ is necessary for normal basal [Ca^{2+}]$_i$ and critical for light-induced [Ca^{2+}]$_i$ increases; but little Ca^{2+} enters through VDCCs. This suggests that [Ca^{2+}]$_o$ enters the cytosol via other routes (e.g. TRP channels) or that the contribution of Ca^{2+} through VGCCs is slight but serves to trigger Ca^{2+}-induced Ca^{2+}-release (CICR) from ER stores.