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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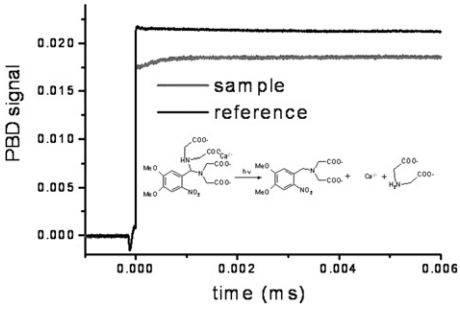
DM-nitrophen is a popular caged calcium compound that allows for a rapid increase in calcium concentration from hundred nanomolar to tens to hundred micromolar level on the microsecond timescale. To fully understand the mechanism of calcium photo-release from DM-nitrophen, we have used photothermal beam 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

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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