

Ca²⁺ nanodomains are shaped by the interplay between Ca²⁺ influx, diffusion and binding to Ca²⁺ buffers, and can be approximated with reasonable accuracy by analytic approximations of quasi-stationary solutions of the corresponding reaction-diffusion equations. Such closed-form approximations help to reveal the qualitative dependence of nanodomain characteristics on buffering and diffusion parameters without resorting to computationally expensive numerical simulations. Although several nanodomain approximations had been developed for the case of buffers with a single Ca²⁺ binding site, most biological buffers have more complex Ca²⁺-binding stoichiometry. Further, Ca²⁺ buffers such as calretinin and calmodulin consist of distinct EF-hand domains, each possessing two Ca²⁺ binding sites exhibiting cooperativity in binding, whereby the affinity of the second Ca²⁺ binding is much higher than the first. While the Rapid Buffering Approximation (RBA) has been recently generalized to such cooperative buffers, its performance is limited by the complex interplay between the condition of slow diffusion required for RBA accuracy, and the slow rate of the first Ca²⁺ binding reaction characterizing cooperative Ca²⁺ binding. To resolve this problem, we extend the recently developed alternative method, the Padé approximation method, to the case of 2-to-1 Ca²⁺ buffers. The Padé approximation interpolates between the short-range and long-range distance-dependence of nanodomain concentration using a rational function Ansatz. We examine in detail the parameter-dependence of the lowest-order Padé approximation accuracy, and show that this method is superior to RBA for a wide range of buffering parameter values. The limitations of the Padé method, in particular its algebraic complexity, are also discussed. Supported in part by NSF DMS-1517085 (V.M)

1190-Pos

New Red Fluorescent Calcium Indicators for Functional Analysis of GPCRs and Ca²⁺ Channel Targets

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The intercellular calcium flux assay is a widely used in monitoring signal transduction pathways and characterizing GPCRs agonists and antagonists. Most common calcium indicators are green fluorescent and difficult for using with GFP and YFP transfected cells or tissues, so red fluorescent calcium indicators are highly desirable. Although Rhod-2 is commonly used for multiplexing with GFP-transfected cells, the higher staining background and cellular localization (mostly in mitochondria) limits its applications and makes the rhodamine-based calcium dyes less sensitive. In this study, two new red fluorescent calcium indicators, Calbryte 590 and Calbryte 630, were developed for monitoring Ca²⁺ flux assays in living cells with Ex/Em= 580/592 nm and Ex/Em=608/624nm, which are red-shifted wavelength than Rhod-2 with no overlap with FITC filters. In this study, the performance of Calbryte 590 and Calbryte 630 was evaluated with different receptor signaling pathways using HEK-293, CHO-M1 and CHO-K1 cell lines with fluorescence microscope, microplate reader and flow cytometer. The fluorescence imaging data showed that Calbryte 590 and Calbryte 630 uniformly bind to cytosolic calcium ion. The microplate assay results showed that unlike Rhod-2, which requires the presence of organic anion transporter inhibitor (e.g. probenecid) to prevent the leakage of the indicators during dye loading, Calbryte 590 and Calbryte 630 requires minimal amount of probenecid and still has around 5 folds of response over background, which is more than twice higher compared to Rhod-2 assay (S/B ~2 folds). Calbryte 630 flow cytometry assay results also showed ~4 folds Ca²⁺ flux change with APC channel. In conclusion, Calbryte 590 and Calbryte 630 are robust red-shifted fluorescent indicators suitable for multiplexing Ca²⁺ flux assay in GFP- and YFP-transfected cells or with other green fluorescent dyes with improved cellular retention, brightness and S/B ratio.

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The Arrhythmogenic E105A CAM Mutation Dysregulates Normal Cardiac Function in Zebrafish by Altering CAM-Ca²⁺ and CAM-RyR2 Interactions

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Calmodulin (CaM) is a multifunctional calcium (Ca²⁺)-binding messenger that directly interacts with the cardiac ryanodine receptor 2 (RyR2), a large transmembrane Ca²⁺ channel that mediates Ca²⁺ release from the sarcoplasmic reticulum to activate cardiac muscle contraction. Genetic studies have reported CaM missense mutations in individuals with history of life-threatening arrhythmogenic heart disorders. A recent clinical report identified a novel, long QT syndrome (LQTS)-associated CaM mutation (E105A), in a child, who experienced an aborted first-episode of cardiac arrest. Herein, to determine the functional consequences of the E105A mutation *in vivo*, we introduced this mutation into human CaM sequence and we injected synthetic mRNA encoding CaM^{WT} and CaM^{E105A} into zebrafish embryos. Although expression of CaM^{WT} and CaM^{E105A} proteins in zebrafish did not affect the normal embryo development, a slight change in the heart morphology was observed, with ~31.5% of the CaM^{E105A}-injected zebrafish exhibiting extended hearts. Furthermore, analysis of the cardiac activity of the zebrafish ventricle revealed that CaM^{E105A} mutant-injected larvae displayed irregular pattern of heart beating in comparison to the median of the CaM^{WT} and control groups, resulting to an increased arrhythmic potential in these embryos. In addition, the average heart rate was significantly increased in this group (~160.5 beats per minute (bpm) vs ~152.5 bpm of control group). *In vitro* Ca²⁺-binding studies revealed that the C-domain of CaM^{E105A} mutant exhibited a ~10-fold reduced Ca²⁺-binding affinity compared to CaM^{WT}. Finally, the functional effect of E105A mutation on RyR2 activity was assessed by a [³H]ryanodine binding assay and suggested that CaM^{E105A} mutant shows a dramatically reduced inhibition of ryanodine binding to RyR2 compared to CaM^{WT}. Our findings suggest that LQTS-associated E105A CaM mutation dysregulates normal cardiac function in zebrafish by altering both CaM-Ca²⁺ and CaM-RyR2 interactions.

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Calcium Channels Contributing to Action Potential Firing and Rhythms in the Circadian Clock

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Mammalian circadian rhythms are timed by suprachiasmatic nucleus (SCN) neurons, which generate a ~24-hour time signal by firing spontaneous action potentials (APs) at a higher frequency during the day versus the night. The roles of Ca²⁺ channels in regulating AP firing in the SCN have not been extensively studied. To identify which Ca²⁺ channels contribute to the circadian regulation of AP frequency, we tested the effects of inhibitors of VGCCs (Cd²⁺), L-(nimodipine, Nim), N-(ω-conotoxinGVIA, GVIA), P/Q-(ω-agatoxinIVA, Aga), R-(Ni²⁺), and T-(TTAP2) type Ca²⁺ channels, ryanodine receptors (dantrolene, Dan) and SERCA (cyclopiazonic acid, CPA) on extracellular day and night AP frequencies from acute SCN slices. Compared to control, daytime AP frequency decreased in Cd²⁺ (-85%), Nim (-28%), GVIA (-65%), Aga (-69%), Ni²⁺ (-46%), Dan (-31%), and CPA (-27%). Nighttime AP frequency also decreased in Cd²⁺ (-63%) but increased in Dan (+113%) and CPA (+125%). The chronic effect of these inhibitors on AP rhythmicity was determined using 3 days of baseline firing and 3 days post-drug application from organotypic SCNs on multi-electrode arrays. Compared to baseline, the percentage of rhythmic recordings decreased in Nim (-16 ± 6%), GVIA (-16 ± 6%), Aga (-29 ± 5%) and Ni²⁺ (-32 ± 10%). Of the rhythmic recordings remaining, the χ² amplitude decreased with Nim (-55%) and Aga (-48%) and relative power (FFT) decreased with Nim (-57%). Period increased in Dan (+5%), although Dan and CPA did not affect other rhythmic parameters. Our data suggests L-, N-, P/Q- and R-TCCs all contribute to daytime AP frequency and circadian AP rhythms. The strongest influence is contributed by LTCCs, consistent with evidence that LTCC currents are diurnally modulated. In contrast, Ca²⁺_i stores contribute to daytime and nighttime AP frequency but only a limited influence on AP rhythmicity is revealed in this study.

Posters: Other Channels

1193-Pos

Functionally Identifying Members of the MscS Superfamily of Ion Channels in Paraburkholderia Membranes

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Bacterial mechanosensitive channels gate in response to tension in the membrane which can be driven by drastic changes in osmolarity. The mechanosensitive ion channel of small conductance (MscS) from *Escherichia coli* gates in response to mechanical force, and is the most well studied member of the MscS superfamily of ion channels. Despite limited genome space, several MscS superfamily members are predicted in each genome. Members of the bCNG