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A Population Density Domain Model for Calcium-Inactivation of L-Type Calcium Channels

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Presenilin-1 Mutants Connected with Familial Alzheimer's Disease affect Activity of Voltage-Gated Calcium Channels

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Familial Alzheimer's disease (FAD) is caused by mutations in presenilin-1 (PS1) gene in approximately 50% of cases. It was found that FAD PS1 mutants disrupt calcium homeostasis in hippocampal neurons disrupting Ca^{2+} storage in the lumen of endoplasmic reticulum (ER). Recently calcium sensors of ER STIM1 were found to negatively regulate the activity of L-type voltage-gated calcium channels. Therefore it was suggested that FAD PS1 mutants could affect the activity of L-type channels in neurons. To study the activity of voltage-gated calcium channels experiments with a patch-clamp technique in whole-cell mode were performed with human neuroblastoma SK-N-SH cell line transfected with PS1 M146V mutant or PS1 WT. Currents were induced by 10 mV voltage steps per 200 ms from −80 to +40 mV. PS1 M146V mutant expression enchased the amplitude of integral current at positive potentials comparing to cells with expression of PS1 WT and untransfected control cells. Currents were found to be blocked by application 10 uM of nifedipine. Knock-down of STIM1 with shRNA abolished the difference between cells with mutant and PS1 WT expressions but in the same time mock shRNA left the difference unchanged. Knock-down of STIM1 was controlled by western-blot of cell lysates. Expression PS1 M146V enhanced the amplitude of calcium entry in mouse hippocampal neurons induced by depolarization with 140 KCl in calcium imaging experiments with Fura2-AM comparing to PS1 WT expressing cells. It was concluded that PS1 M146V mutant connected with FAD affect activity of L-type calcium channels through the STIM1 calcium sensors.

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How CaBPs Prevail over CaM in Modulating Cav1 Channels

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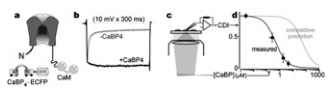
Calcium-binding proteins (CaBPs), a subfamily of calmodulin (CaM)-like proteins, are widely expressed throughout the brain. They are believed to compete with CaM for binding to target molecules, thereby exerting vital modifications of Ca^{2+}/CaM regulation. Yet, deep sequencing of brain reveals CaBP transcript levels 100-10000 times lower than for CaM. How could so little CaBP produce effects in the brain? Here, we examine this fundamental paradox in L-type $Ca_V1.3$ Ca^{2+} channels, whose Ca^{2+}/CaM-dependent inactivation (CDI) can be suppressed by $CaBP_4$. Upon fusion of CaM to $Ca_V1.3$ (**a**), the ultra-high local CaM concentration should occlude $CaBP_4$ access to a common site. Remarkably, separately expressed $CaBP_4$ still eliminates CDI (**b**), arguing for different $CaBP_4$ and CaM sites. FRET assays map these distinct sites, and establish corresponding association constants for binding. This noncompetitive scheme predicts that micromolar $CaBP_4$ should reduce CDI, even with abundant CaM. To test this, we used patch fluorometry to measure CDI while gauging the concentration of fluorescently labeled $CaBP_4$ (**c**). $CaBP_4$ indeed halves CDI by ~0.5 uM (**d**, measured), with abundant CaM throughout. Hence, CaBP molecules may prevail over CaM by utilizing distinct sites on target molecules across the brain.



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Detection of Acute Hypoxia in Cardiac Myocytes using Calcium Channel Activity and O_2-Sensitive Coverslips

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Acute and chronic hypoxias are common diseases that often lead to arrhythmia and impaired contractility. At the cellular level it is unclear whether the suppression of cardiac Ca^{2+} channels ($Ca_V1.2$) results directly from oxygen deprivation on the channel protein or functions through intermediary proteins affecting the channel. To address this question we measured the time-course of suppression of the Ba^{2+}-current (I_{Ba}) through L-type Ca^{2+}-channels while monitoring the concentration of dissolved O_2 in the immediate vicinity of the voltage-clamped cell. Cells were plated onto glass coverslips coated with a fluorescent ruthenium-based O_2-sensor (Ruthenium-tris(4,7-diphenyl-1,10-phenanthroline) dichloride) imbedded in ORMOSIL matrix. using the glass coverslips as a light guide, the O_2-sensor was excited by evanescent illumination at 473 nm from a 400 mW diode laser, while emitted light ($>$ 515 nm) from the para-cellular vicinity was detected by a photomultiplier. using this optical system we verified that our perfusion system was capable of changing the O_2 concentration around the cell within 140 ms ($\tau_{1/2}$). I_{Ba} was activated every 5 sec using 30 ms depolarizing pulses from −50 mV to 0 mV and cells were exposed every third episode to hypoxic solutions lasting 0.25-2.5 sec. Under these conditions the suppression of I_{Ba} increased gradually with the duration of hypoxia. This effect was >80% reversible within 5 sec. These results support the notion that hypoxia has a rapid and direct suppressive effect on the Ca^{2+} channel and are consistent with our previous findings that suggested that the binding of heme oxygenase to the CaM/CaMKII-specific motifs on Ca^{2+} channel may mediate the rapid response of the channel to hypoxia (Rosa *et al.*, 2012, J Physiol, 590:4223-4237). Supported by: RO1 HL 107600.

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A Population Density Domain Model for Calcium-Inactivation of L-Type Calcium Channels

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We present a minimal whole cell model of stochastic domain Ca-mediated inactivation of low-density L-type Ca channels. Our approach avoids the computationally demanding task of resolving spatial aspects of global Ca signaling by using probability densities and associated moment equations to represent heterogeneous local Ca signals [Williams et al. Biophys J. 92(7):2311-28, 2007; Biophys J. 95(4):1689-703, 2008]. using a minimal Markov chain model of an L-type Ca channel, simulated whole cell responses to a two-pulse voltage clamp protocol yield an inactivation function for the whole cell Ca current that is similar to - but may deviate from - that obtained by assuming instantaneous formation and collapse of Ca domains [Sherman, Keizer, Rinzel. Biophys J. 58(4):985-95, 1990]. Parameter studies reveal that when domain Ca formation and collapse are slow compared to channel kinetics (e.g., fast voltage-dependent gating), the population density description is required to accurately represent the dynamics of Ca inactivation of whole cell L-type currents. When channel kinetics are slow, the population density approach agrees with - and thus generalizes - ''instantaneous domain'' models of Ca inactivation.

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Modeling the Structure and Function of L-Type Calcium Channel (Cav1.2) to Understand its Effect in Cardiac Propagation

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The gating dynamics of the voltage gated L-type calcium channel (VGLCC) play an important role in intracellular calcium dynamics and the shape of the cardiac action potential. In this work we focus on modeling the structure of the VGLCC at the atomistic level to elucidate its structure-function relationship. using the crystal structure of the wild type voltage gated sodium channel (NavAb) [i] as the structural template, we propose a model of the Cav1.2 calcium channel. By using Molecular Dynamics simulations of the calcium channel embedded in a model membrane, we have explored the microscopic structure-function relationship of this channel protein. In parallel, we investigated the function of the channel in ventricular propagation dynamics. Our functional goal is to understand the effect of Cav1.2 gating in the ventricular tachycardia to fibrillation transition. With this in mind, we simulated the effect of changing the gating dynamics of the Cav1.2 on two dimensional spiral wave simulations run on a 640x640 grid wherein each node was represented by a Luo-Rudy model of the cardiac cell[ii]. Specifically, we investigated and demonstrated the hypothesis that affecting the time constant of VGLCC inactivation destabilizes spiral wave behavior which is important in the clinical transition from normal rhythm to reentry tachycardia (one spiral) which often degenerates into ventricular fibrillation (multiple spirals). We will present our channel model and its validation to derive hypotheses of how our structure relates to the functional findings.

Payandeh J et.al. Crystal structure of a voltage-gated sodium channel in two potentially inactivated states. Nature. 2012 May 20;486(7401):135-9.
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Hydrolysis of PIP2 is Responsible for the Voltage-Independent Inhibition of Cav2.2 Channels

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GPCRs regulate CaV2.2 channels through both voltage-dependent and -independent inhibition pathways. Gβγ subunits are the effectors of the