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A Continuum Variational Approach to Vesicle Membrane Modeling

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Biological membranes remodel in lipid pore formation, fusion, endocytosis and other processes. Traditionally, continuum membrane mechanics has been used to describe the physics of these remodelings. Membrane mechanics is a conservative, equilibrium theory and so cannot, a priori, describe the time course, flows and dissipations of a real system. Over the past few decades, physical scientists and mathematicians have developed global multi-physics field equations that describe the time course of processes for condensed matter in a thermodynamically consistent way. We use these equations to describe the membrane during lipid bilayer membrane remodelings. We analyze the vesicle membrane and its lipid layers as a bulk continuum variable in a Hamiltonian. The Hamiltonian includes the surface tension and curvature effects of the classical Helfrich model. The representations are, however, more flexible and can readily account for multicomponent systems, inhomogeneities, and changes in topology. Coupling the Hamiltonian to the motion of the aqueous medium with Rayleigh dissipation leads to a complicated, self-consistent system of partial differential equations that is solved numerically. Numerical schemes, designed specifically for this field theory, provide the position, velocity and forces of the fluid–vesicle system at each point in space and time. Classical models assume a specific shape for the vesicle (e.g., a sphere). The assumed shape will occur in the real world, however, only if it is a self-consistent solution of the equations. Our calculations yield values of all key variables and energies over time-the shape is an output. Movies that precisely illustrate the time evolution of the membrane configuration are generated. Changes over time are appreciated visually without reference to the equations–or even to the physics–of the remodeling processes.

PLATFORM Z: Ryanodine Receptors

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Effects of JTV519 (K201) on Na+- and Ca^{2+} Overload-Induced Arrhythmogenic Ca^{2+} Release in Mouse Cardiac Myocytes

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Diastolic Ca^{2+} leak from the sarcoplasmic reticulum (SR) via the ryanodine receptor (RyR2) contributes to arrhythmias. Protein kinase A (PKA) and Ca^{2+}/Calmodulin- dependent protein kinase II (CaMKII) have been involved in SR Ca^{2+} leak by altering RyR2 gating. Under these conditions, JTV519 (K201), a benzothiazepine derivate, has been shown to stabilize the modified RyR2 and reduce diastolic SR Ca^{2+} leak. We tested, whether JTV519 reduces SR Ca^{2+} leak induced by Na^{+}- and Ca^{2+} overload (induced by 100 μM ouabain), i.e. independent of CaMKII signaling. Methods: $[Ca^{2+}]_i$ was measured (Fluo4-AM, confocal) in paced murine cardiomyocytes ±JTV519 (1 μM, >1h preincubation). $[Ca^{2+}]$-transients, diastolic SR Ca^{2+} leak (Ca^{2+} spark (SparkF, in $s^{-1}*pL^{-1}$) and Ca^{2+} wave frequency) and SR $[Ca^{2+}]$ (caffein) were measured ± ouabain (OUAB) and KN93 (CaMKII-inhibitor, 1 μM). Phosphorylation of RyR2 (pSer2814, CaMKII site) was quantified by Western blot. Results: With OUAB, total cellular $[Ca^{2+}]$ increased from 3.3 ± 0.3 to 4.3 ± 0.3 (F/F0, mean ± SE), SparkF increased from 31 ± 20 to 85 ± 30 (both p<0.05). Ouabain did not increase pSer2814, and KN93 had no effect on elevated SparkF with ouabain (89 ± 24), indicating no contribution of CaMKII to increased SR Ca^{2+} leak. JTV519 decreased SparkF (25 ± 4), and Ca2+ waves, but also attenuated the increase in SR $[Ca^{2+}]$ in OUAB (F/F0: 5.2 ± 0.3 with JTV+Ouab vs. 7.7 ± 0.6 OUAB, p<0.05). However, matching cells for equal SR $[Ca^{2+}]$ revealed an SR $[Ca^{2+}]$ - independent effect of JTV519 on SparkF. In contrast, propagation speed of Ca^{2+} waves and the ratio between $[Ca^{2+}]$ transient amplitude and SR $[Ca^{2+}]$ were unchanged.

Conclusion: In conditions not related to CaMKII-mediated alterations of RyR2 gating, JTV519 reduces spontaneous SR Ca^{2+} release, but does not influence propagated Ca^{2+} release or the fraction of SR Ca^{2+} released with each beat.

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AICAR Suppresses Intrinsic Ryanodine Receptor-Dependent Store-Operated Calcium Entry in CHO Cells

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AICAR, an AMP-activated protein kinase (AMPK) agonist, increases the efficiency of murine oxygen metabolism, dramatically increasing exercise endurance (Cell 134:405, 2008). AICAR also prevents heat-induced death in malignant hyperthermia(MH)-susceptible transgenic mice (RyR1-Y522S/WT) without changes in AMPK phosphorylation, and suppresses intracellular Ca^{2+} levels by an unknown mechanism (Lanner JT et al, 2010 Biophysical Society Meeting Abstracts). Store Operated Ca^{2+} Entry (SOCE) restores Ca^{2+} to depleted sarco∴endoplasmic reticulum from extracellular stores. Since azumolene, a drug which inhibits MH, suppresses the RyR1-dependent rise in intracellular Ca^{2+} by inhibiting SOCE rather than Ca^{2+} release, we sought to determine whether AICAR affects Ca^{2+} homeostasis similarly. We compared CHO cells stably transfected with RyR1 (C1148) with wild type (CHO-wt). Single cell Ca^{2+} imaging was performed in Fura-2 AM-loaded cells. Changes in intracellular Ca^{2+} were followed as changes in the ratio λ340/380, and SOCE measured by Mn^{2+}-quenching of Fura-2 fluorescence (λ360). In C1148 cells, AICAR (1 mM, overnight) suppresses Ca^{2+}-release by the RyR1 agonists, caffeine and 4-Chloro-m-Cresol (4-CMC). Western blot and immunofluorescence analysis do not show any change in the expression of RyR1 or STIM1. The elevated SOCE in C1148 cells is largely inhibited by AICAR (1 mM) and virtually completely by the specific SOCE inhibitor BTP2 (1μM). SOCE stimulated by the SR Ca^{2+}-pump inhibitor, CPA (10 μM), was inhibited by BTP2, but not by AICAR. The ER Ca^{2+} store in C1148 cells is much lower than that of CHO-wt, irrespective of AICAR treatment status. AICAR has no significant effect on [^{3}H]ryanodine-binding to skeletal muscle SR vesicles, nor does it have any effect on sarco∴endoplasmic reticulum Ca^{2+}-ATPase activity. We conclude that AICAR, like azumolene, partially inhibits the RyR1-dependent SOCE, but not Ca^{2+}-ATPase-dependent SOCE. These data also suggest that AICAR may be therapeutic in treating MH.

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Novel Mechanisms of CPVT1 in a RyR2-V2475F Knock-In Mouse Model

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Mutations in the cardiac ryanodine receptor (RyR2) have been implicated in Catecholaminergic Polymorphic Ventricular Tachicardia 1 (CPVT1), an arrhythmogenic syndrome triggered by emotional or physical stress in otherwise healthy individuals. At the cellular level, the adrenergic surge during stress presumably induces diastolic SR Ca2+ release, generating a favorable substrate for arrhythmias by the mechanism of delayed afterdepolarization (DAD). Increased sensitivity to luminal [Ca2+], unzipping of domain-domain interactions, and de-regulation by accessory proteins have been reported to contribute to RyR2 dysfunction in CPVT1. We generated a knock-in mouse model of CPVT1 (RyR2-V2475F) and characterized its mechanisms of arrhythmia. Expression of RyR2-V2475F in HEK293 cells induces a unique phenotype, namely, increased activity at diastolic [Ca2+] (pCa 7), which is not seen in other CPVT1 mutations. Mice homozygous for the V2475F mutation have not been detected in >500 mice, suggesting that the mutation causes catastrophic RyR2 dysfunction and embryonic lethality. Heterozygous mice, on the other hand, display increased propensity for premature ventricular complexes, bidirectional ventricular tachycardia and other forms of tachyarrythmias when injected a cocktail of norepinephrine and caffeine that is mostly innocuous in wild-type mice. Heterozygous Langendorff-perfused hearts subjected to adrenergic stimulation and tachypacing are also arrhythmogenic, indicating that the arrhythmias arise from mechanisms intrinsic to the heart and not necessarily from systemic factors. Despite this severe phenotype, ventricular cardiomyocytes isolated from RyR2-V2475F mice display only mild differences in the amplitude and kinetics of intracellular Ca2+ transients when compared to wild-type myocytes, regardless of stimulation frequency and β-adrenergic stimulation. Thus, cellular Ca2+ mishandling in ventricular myocytes of the RyR2-V2475F mice, although significant, does not appear sufficient to support the severe phenotype observed in whole hearts and intact mice. We are therefore investigating other mechanisms that may explain the arrhythmogenicity of heterozygous mice.

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FRET-Based Mapping of Three Structural Domains within the Full-Length Ryanodine Receptor Type 1

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The type 1 ryanodine receptor (RyR1) mediates excitation contraction coupling in skeletal muscle by releasing intracellular calcium in response to cell depolarization. Understanding the structure and conformational dynamics of RyR1 is challenging due to its size (homotetrameric subunit Mr~560 kDa) and its association with numerous regulatory proteins. However, recent X-ray crystallographic studies indicate that a fragment comprised of the first 200 amino acids of RyR1 folds into a compact β-trefoil domain. Based on structural homology with the inositol trisphosphate receptor, the next 400