disorganized T-tubular structure in failing myocytes, which is also known to promote Ca^{2+} release dyssynchrony. Specifically, we observed irregular gaps between adjacent tubules where Ca^{2+} release was markedly delayed, occurring only after Ca^{2+} diffusion from regions where tubules were present. Thus, slowed and dyssynchronous Ca^{2+} release in failing myocytes results from a combination of altered ryanodine receptor function and T-tubule disorganization. We suggest that the sub-population of slow, small amplitude Ca^{2+} sparks in CHF may represent ryanodine receptors which are functionally uncoupled from their neighbours.

555-Pos

Cardiotrophin-1: Another "player" in Cardiac Calcium Handling

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Cardiotrophin-1 (CT-1) is a cytokine member of the interleukin-6 superfamily produced by cardiomyocytes and fibroblasts in the heart, in situations of haemodynamic overload, or in the presence of humoral factors as aldosterone. CT-1 is able to induce hypertrophic growth and dysfunction of cardiomyocytes *in vitro*. Moreover, plasma levels of CT-1 are elevated in patients with cardiac hypertrophy and heart failure (HF) and correlated with the severity of the disease. On the other hand, it is well established that alterations in calcium handling are involved in cardiac dysfunction during HF. However, it is yet unknown whether CT-1 modulates Ca^{2+}handling in cardiomyocytes. Here we analyzed CT-1 effects on $[Ca^{2+}]_i$ handling in rat single cardiomyocytes. The L-type calcium current (I_{CaL}) was registered using whole-cell patch-clamp technique. Intracellular calcium $[Ca^{2+}]_i$ transients and Ca^{2+} sparks were viewed by confocal miscroscopy in cardiomyocytes loaded with the fluorescence Ca^{2+} indicator Fluo-3 AM. Treatment of cardiomyocytes with 1 nM CT-1 for 30 min induced a significant increase in I_{CaL} density compared to control cells (at -10 mV: -16.0 ± 0.9 vs. 11.9 ± 0.7 pA/pF; $P < 0.01$). The activity of ryanodine receptors (RyRs), estimated by Ca^{2+} spark frequency, was significantly increased in cardiomyocytes treated with CT-1 (Ca^{2+} sparks$\cdot s^{-1}\cdot 100\ \mu m^{-1}$: 2.3 ± 0.3 vs. 4.3 ± 0.5; $P < 0.01$). Moreover, we observed that the increase in the total Ca^{2+} spark frequency produced by CT-1 could be attributable to the increased propensity of some clusters of RyR to release Ca^{2+}repetitively. Thus, we conclude that CT-1 is able to alter Ca^{2+} handling in isolated cardiomyocytes, enhancing the Ca^{2+} influx through L-type Ca^{2+} channel and the Ca^{2+}release from sarcoplasmic reticulum through RyRs.

556-Pos

Occurrence of Spontaneous Sparks in Ventricular Myocytes From Junctional and Non-junctional Ryr Clusters

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In isolated rabbit ventricular myocytes, we found a significant number of ryanodine receptor (RyR) clusters that are not associated with the sarcolemma (non-junctional RyRs). The contribution of non-junctional RyR clusters to calcium transients is unclear. Here, we investigated if these non-junctional RyRs are able to produce spontaneous local calcium release events (sparks), and compared the probability of non-junctional versus junctional sparks. We imaged spontaneous sparks in cells loaded with fluo-4 and bathed in Tyrode solution with dextran (Molecular Weight: 10 kDa) linked to Texas Red dye. We evoked spontaneous sparks using field stimulation in the presence of 1 μM isoproterenol and 4 mM calcium. After 5 stimuli applied with a frequency of 0.5 Hz, we simultaneously imaged the sarcolemma and spontaneous sparks using a line scan confocal microscope (Biorad MRC-1024). Furthermore, a 3D image stack of the Texas Red associated signal was acquired to identify the sarcolemma including the transverse tubular system. We classified sparks as non-junctional if their distance to the sarcolemma is larger than 1 μm. All other sparks were assumed to be junctional. In measurements on 12 isolated cells, 38 sparks (51%) were identified as non-junctional, 36 (49%) as junctional. Our measurements clearly demonstrate that non-junctional RyR clusters are able to release calcium and produce spontaneous sparks. We expect that our approach for distinguishing between non-junctional and junctional sparks underestimates the number of non-junctional sparks. If this is true, the probabilities of the types of sparks are similar to probabilities of the two types of RyR clusters identified in related immunolabeling and microscopic studies. This would suggests that spark generation probability of RyR clusters does not depend on their type.

557-Pos

Alteration of Ryanodine Receptor-Mediated Calcium Release in Heart Failure

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The decrease in contractility in heart failure associates with impaired cellular Ca^{2+} homeostasis that is in part due to altered ryanodine receptor (RyR) function. We studied properties of sarcoplasmic reticulum (SR) Ca^{2+} release in normal and failing rabbit ventricular myocytes using simultaneous measurements of cytosolic ($[Ca^{2+}]_i$) and intra-SR free Ca^{2+} ($[Ca^{2+}]_{SR}$). At a given SR Ca^{2+} content, fractional SR Ca^{2+} release during action potential stimulation was higher in failing than nonfailing myocytes, suggesting increased sensitivity of RyRs in heart failure. In permeabilized myocytes, SR Ca^{2+} content and Ca^{2+} spark frequency were decreased in heart failure, while Ca^{2+} spark amplitude was similar between failing and nonfailing myocytes. To compare these two groups further, SR Ca^{2+} content was experimentally decreased in nonfailing myocytes to the level observed in failing myocytes using SERCA inhibition. When SR Ca^{2+} content was matched, both Ca^{2+} spark frequency and amplitude were markedly increased in failing myocytes, showing that RyRs are more sensitive to release activation. By monitoring $[Ca^{2+}]_{SR}$ during Ca^{2+} sparks, we also observed that the $[Ca^{2+}]_{SR}$ level for spark termination was significantly lower in myocytes from failing hearts. Because Ca^{2+} sparks are a major contributing factor to diastolic SR Ca^{2+} leak, we compared the properties of SR Ca^{2+} leak in normal and failing myocytes. In failing myocytes SR Ca^{2+} leak was significantly faster, particularly at high $[Ca^{2+}]_{SR}$ where Ca^{2+} sparks are the predominant pathway for SR Ca^{2+} leak. These data show that during the progression of heart failure, modifications to RyRs alter both activation and termination of local SR Ca^{2+} release events. At a given SR Ca^{2+} content these effects may increase fractional SR Ca^{2+} release and preserve contractility during systole, however at the cost of increased diastolic SR Ca^{2+} leak and SR depletion.

558-Pos

Abnormal Intra-Store Calcium Handling and Arrhythmogenesis in Heart Failure

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Heart failure (HF) patients are known to have increased susceptibility to ventricular arrhythmias. Although abnormal intracellular calcium (Ca) cycling is recognized as an important contributor to the pathogenesis of ventricular arrhythmias, the specific cellular and molecular mechanisms of these arrhythmias remain to be defined. The objective of present study was to investigate the sub-cellular mechanisms of Ca-dependent arrhythmia using time-resolved Ca imaging in the cytosolic and sarcoplasmic reticulum (SR) luminal compartments and the patch-clamp technique in a canine model of tachypacing-induced HF. When rhythmically paced in the presence of the β-adrenergic agonist, isoproterenol, HF myocytes displayed a higher frequency of diastolic Ca waves than control myocytes. In both HF and control myocytes, diastolic Ca waves occurred when [Ca]SR rose above a certain threshold level, which was significantly lower in HF than in control myocytes. Ca signaling refractoriness determined as the time delay between systolic SR Ca depletion and Ca wave initiation was significantly reduced in HF myocytes. Electrical and Ca signaling activities exhibited several distinct potentially arrhythmogenic patterns, including: 1) delayed afterdepolarizations and extrasystolic action potentials (APs) linked to diastolic spontaneous Ca waves; 2) intermittent prolongations of AP duration associated with pre-systolic spontaneous Ca waves and post-systolic triggered Ca waves; and 3) disorganized release uncoupled from myocyte electrical activity. The level of [Ca]SR threshold for spontaneous Ca waves and the time to attain the threshold during the pacing cycle were critical in determining the type of arrhythmogenic abnormality. These experiments suggest a common mechanistic framework for apparently different arrhythmic phenotypes and provide new insights into the relationship between abnormal Ca release and arrhythmogenesis in HF.

559-Pos

Impaired Function of Cardiac Ryanodine Receptors in An Experimental Model of Metabolic Syndrome

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Metabolic syndrome (MS) has become a global epidemic. In Mexico, the prevalence of MS has increased in the last 10 years together with obesity and type-2

diabetes. MS is a serious health problem due to its related cardiovascular disorders: hypertension and heart failure. The latter is among the major causes of death in México. The molecular mechanisms responsible for MS are unclear but could be related to anomalies in cardiac excitation-contraction coupling (E-C coupling). The cardiac Ca^{2+} channel, the Ryanodine Receptor (RyR2), is a key macromolecular complex that participates in releasing Ca^{2+} from internal stores and is centrally involved in the modulation of cardiac E-C coupling. Our aim was to examine alterations in the expression level, phosphorylation status, Ca^{2+} sensitivity and *in situ* function (Ca^{2+} sparks and Ca^{2+} transients) of RyR2 that could explain the cardiac dysfunction associated with MS.
MS was induced in our rat model by adding commercially refined sugar (30% sucrose) to their drinking water. The sucrose-fed rats became overweight with an increased accumulation of waist fat and also developed hypertension. Our [^{3}H]ryanodine binding data show that functional RyR2 are decreased in MS rat hearts with slight but insignificant changes in Ca^{2+} sensitivity. Western Blot analysis confirmed that MS did not alter the phosphorylation status of RyR2 at Serine-2809 normalized with respect to total RyR2. A significant decrease in Ca^{2+} spark frequency was found in isolated Fluo-3 loaded cardiomyocytes of MS rats. In addition Ca^{2+} transients elicited at frequencies of 0.5, 1, and 2 Hz were also impaired, suggesting a diminished Ca^{2+} cycling condition in MS cardiomyocytes.
Overall, the decreased RyR2 expression together with the impaired RyR2 function could account for the reported poor overall cardiac outcome found in this animal model of MS.

Peptide & Toxin Ion Channels

560-Pos
Effect of Dipole Modifying Agents on the Surfactin Induced Conductance of Planar Lipid Bilayers
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We studied effects of compounds changing membrane dipole potential on membrane conductance induced by antimicrobial lipopeptide surfactin. Surfactin added on both sides of an artificial lipid bilayer from diphytanoyl phosphocholine in 1 M KCl (pH 6.5) produces an increase of the membrane conductance as a result of ion channel formation. Increasing a membrane dipole potential adding RH 421 to the bilayer bathing solution (10 μM , both sides) leads to ~40 times increase of a steady-state conductance. At the same time, addition of phloretin (20 μM), known to decrease the dipole potential, results in decrease of the surfactin-induced conductance by ~30 times. We note, that the effects of dipole modifiers on the surfactin-induced membrane conductance are clearly opposite to the effects observed with the same modifiers in case of syringomycin E-induced conductance of lipid bilayers [Ostroumova et al., Langmuir, 2008]. As we suggested earlier, the influence of dipole modifiers on syringomycin activity may be related to a promotion/retardation of a movement of positively charged syringomycin molecules in the direction of membrane hydrocarbon core. In contrast to syringomycin, surfactin is negatively charged. Hence, one can expect an inversion of the effect of dipole modifiers in case of surfactin. The obtained results are in agreement with the model proposed in [Ostroumova et al., Langmuir, 2008].
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561-Pos
Effect of Antibacterial Peptide Indolicidin on the Membrane Permeability: Carrier Mechanism Versus Pore Formation
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It is generally accepted that the predominant mechanism of action of antimicrobial peptides is the permeabilizaiton of bacterial membranes via formation of aqueous pores. It has been shown in the present work that the main mechanism of carboxyfluorescein (CF) leakage from lipid vesicles induced by antimicrobial peptide indolicidin is not pore formation but rather translocation across the membrane of the complexes of the dye and the peptide, i.e. indolicidin functions as a carrier of organic anions. This conclusion was made after observation of strong inhibition of CF leakage by other organic anions (such as fatty acids) and also inability of indolicidin to induce leakage of glucose and positively-charged doxorubicin. Besides, formation of complexes of indolicidin with pyrenebutanic acid was directly observed by fluorescent assay. The mode of action proposed here for indolicidin can be related to that previously postulated for oligoarginine derivatives which are able to transport organic anions across liposomal and bulk phase membranes [Sakai N. et al., ChemBioChem. 2005, 6:114-122]. The newly identified mechanism may be involved in bactericidal action of indolicidin either directly or indirectly through induction of leakage of important anionic metabolites leading to regulatory disfunction.

562-Pos
Molecular Action Mechanism of Amphotericin B and Structural Analogs on Biological Membranes
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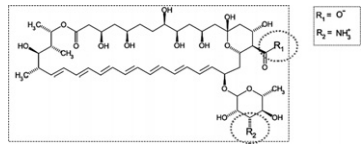
Amphotericin B is a polyene antifungal drug, often used intravenously for systemic fungal infections. It is believed that AmB associates with ergosterol, the main component of fungal cell membranes, forming a transmembrane channel that leads to K+ leakage and fungal cell death. United atom Molecular Dynamics simulations where used to study the mechanism of action of AmB, and other structural analogs, on a POPC and ergosterol bilayer (3:1), varying toxins concentration. It is shown that toxins aggregate first in solution before adsorbing into the membrane, both at low and high concentrations. Electrostatic properties of AmB play an important role in toxicity and sterol selectivity in comparison with AmB's structural analogs.



563-Pos
Divalent Cations Regulate Pore Formation of Synthetic, Naturally Occurring Alamethicin and Selected Analogs
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The biophysical characteristics of synthetic, naturally occurring peptides forming membrane-spanning channels were investigated by using isolated rod outer segments (OS) of frog, recorded in whole-cell configuration. The peptides were applied to (and removed from) the OS in ~50 ms with a computer-controlled microperfusion system. Once blocking the main OS endogenous conductance (the cGMP channels) with saturating light, the OS membrane resistance was mostly >1 GOhm. In symmetric K or Na, 1 μM synthetic alamethicin F50/5 produced a current after ~0.21 s from the solution exchange (called *Delay*), that activated monoexponentially (time constant τ_a~0.26 s) to a maximal amplitude (I_{max}) of ~700 pA. Peptide removal caused the current to return to 0, with a non-measurable *Delay*, and again monoexponentially (time constant τ_d~0.31 s), showing the full reversibility of the permeabilization process. I_{max}, *Delay*, τ_a, and τ_d of current produced by 1 μM of [L-Glu(OMe)^{7,18,19}] (where the Gln 7,18,19 were substituted with side-chain esterified Glu residues) were respectively similar, ~8-fold, ~16-fold, and ~6-fold larger than those of alamethicin F50/5. For both peptides, the current-to-voltage characteristics (obtained with voltage ramps) showed a strong inward rectification at early times of application; current was carried equally well by monovalent and divalent cations. However, activation kinetics accelerated more than 100-fold if external Na or K was substituted with an equiosmolar amount of Ca in the case of F50/5, but up to 10-fold in the case of [L-Glu(OMe)^{7,18,19}]; *Delay* and τ_d were not significantly affected by divalent cations. Similar results were preliminarily obtained in the presence of Mg or Mn, indicating that the effect of divalent cations was not due to a change in the surface charge density of plasma membrane, but to an increase of probability of pore formation.