3698-Pos
Cardiac Action Potential Model with Spatial Subcellular Calcium Cycling and Physiological Transmembrane Currents
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Calcium (Ca) is a key player in excitation-contraction (EC) coupling in the cardiac myocyte. When Ca enters through L-type Ca channels, it opens the ryanodine receptor (RyR) channels on the sarcoplasmic reticulum (SR) which stores a large amount of Ca (Ca induced Ca release). There are about 20,000 SR units and each RyR can sense only the local Ca concentration. Most computer cardiac action potential models use a single Ca concentration, which limits accurate modeling of intracellular Ca cycling. This is because the RyRs do not sense the average Ca concentration of the whole cell. Different aspects limit the utility of various models for investigation of intracellular Ca cycling related phenomena such as delayed afterdepolarizations (DADs) and catecholaminergic polymorphic ventricular tachycardia (CPVT) (since Ca concentrations in these phenomena are strongly heterogeneous within the cell). In order to investigate these phenomena, we developed a mathematical action potential model based on the spatial Ca model by Restrepo et al and physiological transmembrane currents by Shannon et al and Mahajan et al. In order to have more natural sparks, we increased the number of grid points for Ca diffusions from the model by Restrepo et al. Then we tuned the model to exhibit physiological action potential duration (APD) and Ca transients for long and short pacing cycle lengths (PCL). At short PCL, this hybrid model shows both voltage driven alternans due to steep APD restitution and Ca driven alternans due to luminal Ca regulation-mediated mechanism. In addition to alternans, this model exhibits Ca waves and DADs. This mathematical model of the cardiac myocyte provides a sort of minimal model to explore the spatio-temporal aspects of wave initiation, propagation and DAD induction related to normal and pathophysiological conditions.

3699-Pos
Role of Microscopic Heterogeneities in the Organization of Cardiac Sarcoplasmic Reticulum in the Genesis of Calcium Alternans
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Calcium (Ca) alternans are recognized as important contributor to the development of cardiac arrhythmias. It has been proposed that a steep dependence of Ca release from the sarcoplasmic reticulum (SR) on SR Ca content may be responsible for the generation of Ca alternans. The present study investigated the subcellular mechanisms of the steep SR Ca release-load relationship and its role in generation of Ca alternans in canine cardiac myocytes using spatially-resolved Ca imaging in the cytosolic and SR compartments. Analysis of the first derivative of the fluorescence signal of cytoplasmic Ca revealed that the linear portion of Ca release-load relationship, observed at low levels of SR Ca, was associated with a single component of Ca release, while the steep portion, observed at higher SR Ca content, coincided with an appearance of a second release component. The second release component was associated with a significant increase in the amplitude and the recovery time of the SR Ca depletion signal. Spatial analysis of the cytoplasmic Ca signal indicated that the first component of Ca release was more pronounced in t-tubule dense regions. In contrast, the second component of the Ca release was more prominent in regions with sparse t-tubule. Periodic stimulation of voltage-clamped myocytes in the presence of the oxidizing agent DTDP revealed that the magnitude of the alternans directly corresponded to beat-to-beat variations in the amplitude of the second component of Ca release during successive beats. These results suggest that the steep portion of SR Ca release-Ca load relationship is associated with an activation of a spatially distinct group of Ca release sites. These findings provide new insights in the molecular and subcellular mechanisms responsible for the generation of arrhythmogenic Ca alternans.

3700-Pos
Activation of Reverse Na+-Ca2+ Exchange by the Na+ Current during an Action Potential Augments the Calcium Transient: Evidence from NCX Knockout Mice
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The hypothesis that Na+ influx during the action potential activates reverse Na+-Ca2+ exchange (NCX) and subsequent entry of trigger Ca2+ is controversial. To test this hypothesis, we monitored intracellular Ca2+ with fura-2 before and after selectively inactivating INa just prior to a simulated action potential in patch clamped ventricular myocytes isolated from adult wild-type (WT) and NCX knockout (KO) mice (Circ Res.2004;95:604-611). We inactivated INa using a slow ramp prepulse to −45mV to avoid rapid Na+ influx. In WT cells, inactivation of INa decreased the Ca2+ transient amplitude by 52.6 ± 5.3% ($p<0.001$, n=14) and reduced its release flux (measured as the maximum rate of rise) by 47.0 ± 4.6% ($p<0.001$, n=14). There was no effect on diastolic Ca2+. In striking contrast, Ca2+ transients in NCX KO myocytes were unaffected by the presence or absence of INa (n=5). We next investigated the effect of INa on the extent of transsarcolemmal Ca2+ influx in WT and KO myocytes after depleting SR Ca2+ using thapsigargin and ryanodine. In WT cells, inactivation of INa decreased Ca2+ influx by 37.8 ± 6.0% and reduced the flux of Ca2+ entry (maximum rate of rise) by 30.6 ± 7.7%. This effect was absent in the KO cells. We conclude that Na+ current and reverse NCX modulate Ca2+ release in murine WT cardiomyocytes by augmenting the pool of diadic cleft Ca2+ that triggers ryanodine receptors. This may be an important mechanism for Ca2+ release and therefore regulation of contractility in the heart. The use of cardiac-specific NCX KO myocytes provides a unique opportunity to demonstrate a role for NCX in the initiation of cardiac excitation-contraction coupling.

3701-Pos
Modulation of L-Type Calcium Current by Calcium-Dependent Mechanism in Trout Ventricular Myocytes
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In cardiac myocytes, Ca has a dual role upon of L-type calcium current (ICa) by either inactivating it (calcium-dependent inactivation, CDI) or facilitating it (calcium-dependent facilitation, CDF). In fish cardiac myocytes, it is unclear whether both Ca modulations exist. This study addresses this point. Trout ventricular myocytes were enzymatically isolated. ICa was recorded using whole cell patch clamp with Na- and K-free solutions to avoid contaminating currents. With a low concentration of a slow Ca buffer (EGTA 2mM) in the pipette solution, ICa inactivated slowly (compared to mammalian cardiac myocytes): the time to reach 37% of peak current (T37) was 26.2 ± 2.4 ms (mean ± SEM, n=14). CDF was absent in all cells studied. When a fast Ca buffer (BAPTA 10 mM) was present in the pipette solution, ICa decay was similar to the decay in the presence of EGTA (T37: 25.4 ± 1.5 ms, NS, t-test, n=9) and CDF was absent (n=9). We quantified the relative contribution of CDI and sarcoplasmic reticulum (SR) CDI according to our published method, and estimated that CDI represents ~39% of total ICa inactivation, and that SR Ca release causes ~12% of CDI. We conclude that in fish myocytes CDI play a role in ICa modulation but CDF is absent.
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3702-Pos
Osmolarity Modulation of Ca2+ Entry in Depleted and Non-Depleted, CPA Poisoned, Murine Skeletal Muscle Fibers
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A myoplasmic $[Ca^{2+}]_{myo}$ increase takes place in enzymatically dissociated murine FDB fibers, after depletion of their SR Ca^{2+} stores in the presence of CPA and absence of external Ca^{2+}. This $[Ca^{2+}]_{myo}$ increase, activated by external Ca^{2+} and presumably due to SOCE activation, occurs at a rate of 1.1 ± 0.1 nM/s (n=6), and reaches $[Ca^{2+}]_{myo}$ levels above 203 ± 14 nM in141 ± 8 s. Interestingly a smaller $[Ca^{2+}]_{myo}$ increase, also occurs in fibers not previously depleted, albeit at a slower rate of 0.5 ± 0.1 (n=6) reaching levels of about 153 ± 32 nM in 122 ± 27 s. In both, depleted and non-depleted fibers, hypoosmotic solutions (250 mOsm) stop and even reverse this Ca^{2+} increase. Reexposure to the isoosmotic (300 mOsm) medium reactivates Ca^{2+} entry, causing $[Ca^{2+}]_{myo}$ to increase at a faster rate of 12.6 ± 3.7 and 11.5 ± 5.8 in depleted and non-depleted fibers respectively. In both cases, repeated osmolarity change cycles cause a progressive decrease of the effect. Reputed SOCE inhibitors as KB-R7943, 2-APB and Gd^{3+} have similar effects on $[Ca^{2+}]_{myo}$ increases in depleted and non-depleted fibers, suggesting a mechanism similar to SOCE in both cases, but not necessarily depending on complete SR store depletion.
Preliminary experiments with depleted fibers indicate that the sensitivity of the SOCE mechanism to hypoosmotic solutions is lost after treatment with Ryanodine 50 μM during 600 s.
The results suggest that in mouse muscle fibers: 1) SR Ca^{2+} store depletion is not an absolute requirement for SOCE activation; 2) SOCE activation is affected by fiber volume changes and 3) Ryanodine receptors may interact with the machinery of SOCE. (FONACIT G-2001000637).

3703-Pos
Properties of Store Operated Ca^{2+} Entry in Malignant Hyperthermia Susceptible Human Skeletal Muscle Fibres
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Experiments were carried out to investigate the possibility that store operated Ca^{2+} entry (SOCE) may be triggered by volatile anaesthetics in malignant hyperthermia susceptible (MHS) human skeletal muscle. Samples of *vastus medialis* muscle were obtained from patients undergoing assessment for malignant hyperthermia (MH) susceptibility using the standardised *in vitro* contracture test. All experiments were performed with institutional Research Ethics Committee approval and informed patient consent, according to the Declaration of Helsinki. Single fibres were mechanically skinned and confocal microscopy used to detect changes [Ca^{2+}] within the re-sealed t-tubules (with fluo-5N) or within the cytosol (with fluo-3). In normal fibres (MHN), exposure to 0.5 mM halothane failed to trigger SR Ca^{2+} release, or to induce depletion of t-tubule Ca^{2+} (n=8). However, in MHS fibres, 0.5 mM halothane induced both SR Ca^{2+} release and a rapid depletion of t-tubule Ca^{2+}, consistent with SOCE (n=8). In ~20% of MHS fibres, SR Ca^{2+} release took the form of a propagating Ca^{2+} wave and this was associated with a corresponding SOCE wave of t-tubule Ca^{2+} depletion. In MHN fibres, both SR Ca^{2+} release and SOCE could be induced by 0.5 mM halothane when the cytosolic [Mg^{2+}] was decreased to 0.2 mM (n=6). In MHS fibres, SOCE was potently inhibited by inclusion of a STIM1 blocking antibody within the re-sealed t-tubules (n=6). These data suggest (i) that in MHS fibres the degree of SR Ca^{2+} depletion induced by a clinically relevant level of volatile anaesthetic is sufficient to induce SOCE and (ii) that STIM1 located within the sarcolemma modulates SOCE.

3704-Pos

Passive Activation of Store-Operated Ca2+ Entry in Myotubes Depends on the Rate of RyR1-Dependent Ca2+ Leak

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In spite of extensive studies of store operated calcium entry (SOCE), the detailed mechanism of SOCE activation in skeletal muscle remains largely unknown. We recently reported that STIM1-Orai1 coupling is required for SOCE activation in myotubes. However, other proteins that control sarcoplasmic reticulum (SR) Ca2+ content may also contribute to SOCE activation. We hypothesized that passive SOCE activation in skeletal muscle depends on the rate of SR Ca2+ leak through the type 1 ryanodine receptor (RyR1). To test this hypothesis, we conducted a series of whole-cell patch-clamp measurements of SOCE current (ISOC) in myotubes obtained from normal and RyR1-null (dyspedic) mice. Myotubes were bathed in external solution containing (in mM): 138 TEA-methanesulfonate, 10 CaCl2, 10 HEPES, 1 MgCl2, 0.1 nifedipine, pH 7.4. The internal patch pipette solution contained (in mM): 140 mM Cs-methanesulfonate, 10 HEPES, 20 Na-EGTA, 4 MgCl2, pH7.4. SOCE was activated by passive SR Ca2+ depletion following intracellular dialysis with 20 mM EGTA. ISOC in myotubes exhibited many hallmarks of SOCE including strong inward rectification and inhibition by La3+, Gd3+, BTP-2, and 2-APB. ISOC current density at −80 mV was significantly ($p<0.01$) larger in normal myotubes (1.05 ± 0.09 pA/pF, n = 33) compared to that from dyspedic myotubes (0.74 ± 0.07 pA/pF, n = 18). Moreover, the speed of ISOC activation was slower in dyspedic myotubes. Specifically, the time to 10%, 50%, and 90% maximal activation were 4.95 ± 0.65 s, 15.3 ± 1.4 s, and 53.9 ± 7.5 s (n = 19), respectively, in normal myotubes and 67.5 ± 11.1 s, 114 ± 13 s, and 159 ± 17 s (n = 18), respectively, in dyspedic myotubes. These results indicate that RyR1 Ca2+ leak promotes passive SOCE activation.

3705-Pos

Expression of Functional Transgenic Alpha1s-DHPR Channels in Adult Mammalian Skeletal Muscle Fibers

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Plasmids encoding for two variants of the α1sDHPR, tagged at the N-terminal with EGFP, were transfected into adult FDB muscles by *in vivo* electroporation. The wildtype variant of the channel (EGFP-α1sDHPR-*wt*) was rendered insensitive to dihydropyridines by site-directed mutagenesis (EGFP-α1sDHPR-*T935Y*). Standard and TPLSM fluorescence microscopy demonstrated that both variants were similarly expressed with high efficiency and targeted to the surface and TTS membranes of the muscle fibers. Functional evaluation of the efficiency of transgenic expression was carried out by characterizing Ca^{2+} currents and SR Ca^{2+} release in single fibers enzymatically isolated from transfected muscles. The fibers were voltage-clamped using a 2-microelectrode configuration and dialyzed internally with solutions containing 30 to 70 mM Cs-EGTA and 20 to 70 mM Cs-MOPS; they were externally bathed with isotonic TEA-Cl containing 2-12 mM Ca^{2+}. Na and Cl currents were blocked with TTX and 9-ACA, respectively. The maximal Ca^{2+} conductance (gCa_{max}), measured in 12 mM Ca^{2+}, was 0.40 ± 0.04 (mean ± SD, n=6) in control fibers isolated from non-transfected animals. This parameter was not significantly different in fibers expressing EGFP-α1sDHPR-*wt* channels (0.42 ± 0.07, n=6). In contrast, fibers expressing EGFP-α1sDHPR-*T935Y* reported a significantly smaller gCa_{max} of 0.27 ± 0.02 (n=5). Interestingly, after treatment with 1 μM of the specific DHPR blocker NP-200, the residual conductance was <5% in control and EGFP-α1sDHPR-*wt* transfected fibers, but 30-70% in fibers transfected with EGFP-α1sDHPR-*T935Y*. Our results suggest that, in adult skeletal muscle fibers, the functional expression of transgenic DHPR channels is done mostly at the expense of the expression of their endogenous counterparts. Supported by NIH grants Supported by NIH grants AR07664, and AR054816.

3706-Pos

An Important Fraction of the Mammalian Skeletal Muscle Chloride Conductance is Located in the Transverse Tubules

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The actual density of chloride (ClC1) channels in the surface and transverse tubular system (TTS) membranes of mammalian skeletal muscles is still unknown. To investigate this issue, we simultaneously recorded fluorescence signals and chloride currents (ICl) in enzymatically dissociated FDB muscle fibers, stained with the potentiometric indicator di-8-ANEPPS, and voltage-clamped using a 2-microelectrode configuration. The external solution contained (in mM) 150 TEA-Cl, 15 CsMOPS, 2 $CaCl_2$, 0.5 $CdCl_2$, and 200nM TTX. Internally, the fibers were equilibrated with a solution containing 60 CsCl, 40 CsEGTA, 40 CsMOPS, and 5 $MgCl_2$ and voltage-clamped at the chloride equilibrium potential (−20mV). gCl was maximally activated by a pulse to +60mV (150ms) and its voltage-dependence calculated from 9-ACA-sensitive tail currents (measured at the onset of a pulse to −100mV) after 200ms test pulses (−100 to +80mV in amplitude). Boltzmann distributions fitted to the data (n=8) yielded: gCl_{max}=-2.1 ± 0.4 S/F, or 8.1 ± 1.5 mS/cm^2; $V_{1/2}$=73 ± 11 mV and k=24 ± 4 mV. The amplitude (in detaF/F) of di-8-ANEPPS fluorescence transients recorded at the onset of the test pulses were plotted as a function of the pulse amplitudes. In the presence of 9-ACA, the deltaF/F vs. voltage relationship was linear over the entire range of pulse amplitudes explored (slope= −0.124 ± 0.015 /100mV), whereas in the presence of ICl the slope of the linear dependence was less steep. For hyperpolarizing pulses, associated with large instantaneous inward currents, the slope was −0.099 ± 0.03 /100mV; for depolarizing pulses (smaller positive currents) the slope was −0.11 ± 0.025 /100mV. The differential attenuation of the average TTS voltage change in the presence of ICl was predicted by a radial cable model provided that ~30% of the total gCl was in the TTS. Supported by NIH grants Supported by NIH grants AR07664, and AR054816.

3707-Pos

Skeletal Muscle Fibers of Cold-Acclimated Mice Display Increases in Basal Calcium, Mitochondrial Content and Fatigue Resistance

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Mammals initially generate heat by repetitive muscle activity (shivering) when exposed to a cold environment. Shivering can later be replaced by heat generated in brown adipose tissue by activation of uncoupling protein-1 (UCP1). Interestingly, adaptations in skeletal muscles of cold exposed animals are similar to those obtained with endurance training. We studied the function of non-shivering flexor digitorum brevis (FDB) muscles of wild-type (WT) and UCP1-KO mice kept at room temperature (24°C) or cold-acclimated (4°C) for 4-5 weeks. Myoplasmic free [Ca^{2+}] ([Ca^{2+}]$_i$; measured with indo-1) and force were measured under resting conditions and during fatigue induced by repeated tetanic stimulation in intact single fibers. We observed no differences between fibers from WT and UCP1-KO mice. On the other hand, muscle fibers from cold-acclimated mice showed increases in basal [Ca^{2+}]$_i$ (~50%), tetanic [Ca^{2+}]$_i$ (~40%), and SR Ca^{2+} leak (~four-fold) as compared to fibers from room-temperature mice. Muscles of cold-acclimated mice also showed increases in expression of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), citrate synthase activity (reflecting increased mitochondrial content), and fatigue resistance. In conclusion, cold exposure induces changes in FDB muscles similar to those observed with endurance training and we propose that increased basal [Ca^{2+}]$_i$ has a key role in these adaptations.

3708-Pos

Allele Specific Gene Silencing in Autosomal-Dominant Skeletal Myopathies

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Central Core Disease (CCD) and Malignant Hyperthermia (MH) are linked to single amino acid substitutions in the skeletal muscle Ca^{2+} release channel, the type 1 ryanodine receptor (RyR1). We focus on two autosomal dominant (AD)