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.
Supported by the Welcome Trust.

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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