Increased myofilament Ca^{2+} sensitivity is associated with an increased susceptibility for arrhythmias, as previously demonstrated in mice expressing Ca^{2+} sensitizing troponin T (TnT-I79N) mutations and after acute application of the Ca^{2+} sensitizer EMD57033. We hypothesized that the arrhythmia risk is increased due to altered regulation of connexin43 (Cx43), subsequently leading to a slowing of cardiac conduction.

Methods: Fast (longitudinal) and slow (lateral) conduction velocity (CV) was calculated using epi-fluorescence maps from isolated hearts by plotting local CVs against orientation. Phosphorylated Cx43 (P1, P2) from these hearts migrated slower in SDS PAGE and at least three distinct bands could be separated (P0,P1,P2).

Results: The lateral CV, but not the longitudinal CV, was significantly reduced in TnT-I79N mice compared to control (16.9 ± 0.8 cm/s ($n = 11$) vs. 21.5 ± 1.4 cm/s ($n = 11$) respectively, $p<0.05$). As a direct consequence the anisotropy of conduction (fast/slow) was increased in TnT-I79N hearts (to 3.2 ± 0.2 vs 2.3 ± 0.1 in control). This change in CV was associated with decreased Cx43 phosphorylation (un-phosphorylated P0-Cx43 increased to $146 \pm 20\%$ of control, $p<0.05$). Blebbistatin, a Ca^{2+} desensitizer and contractile uncoupler, prevented ventricular arrhythmias in TnT-I79N hearts. Strikingly, blebbistatin also prevented the increase in anisotropy and the decrease in Cx43 phosphorylation, while it had no effect in control hearts. Conversely, acutely increasing Ca^{2+} sensitivity with EMD decreased Cx43 phosphorylation (P0-Cx43 $491 \pm 147\%$, $n = 4$, $p< 0.05$) and rapidly slowed lateral conduction velocity (to 18.2 ± 1.3 vs 22.9 ± 1.1 cm/s in control, $p<0.05$) thereby increasing anisotropy (to 2.64 ± 0.1 vs 2.17 ± 0.07 in control) and rendered control hearts susceptible to arrhythmia induction.

Conclusion: These data suggest that decreased Cx43 phosphorylation and increased conduction anisotropy is at least in part responsible for the increased arrhythmia susceptibility associated with myofilament Ca^{2+} sensitization.

1745-Pos

Electron-Conformational Model of SR-Based Ca^{2+} Clock Mode

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Most of the calcium that activates cardiac contraction comes from the sarcoplasmic reticulum (SR) from where it is released through the Ryanodine Receptors (RyRs). It is well known that the SR overload results in the release of Ca from the SR in the form of waves driving some cardiac arrhythmias. Recently it has been experimentally documented that the isolated SR is capable to spontaneously and rhythmically release Ca^{2+} (SR-based Ca^{2+} clock). This self-sustained intracellular Ca^{2+} oscillator contributes substantially to the late phase of the diastolic depolarization of cardiac pacemaker cells under normal physiological conditions. Interaction of ''a surface membrane oscillator'' and ''an internal oscillator'' with ''cycles of Ca^{2+} uptake and release by the SR'' can drive normal cardiac automaticity. To describe the SR-based Ca^{2+} clock mode we propose a simple, physically-reasonable electron-conformational (EC) model for the RyR and present a theory to describe the RyR lattice dynamics. Each RyR is modelled with a single open and closed electronic state. In addition to the fast electronic degree of freedom, RyR channels are characterized by a slow classical conformational coordinate, which specifies the RyR channel conductance. The RyR gating implies a conformational Langevin dynamics, Ca^{2+}-induced electronic transitions, quantum tunneling and thermal transitions. The cooperativity in the RyR lattice is assumed to be determined by the inter-channel conformational coupling. Model simulations of the of 11x11 RyR cluster revealed different regimes depending on the cis- and trans-Ca concentrations and parameters of EC-model. The SR overload is shown to result in RyR lattice auto-oscillations with spontaneous RyR channel openings and closures. We have studied this Ca^{2+} clock mode, in particular, its stability, under different model suggestions as regards the RyR conformational potential (diabatic and adiabatic regimes), EC-model parameters.

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1746-Pos

Design of a Fluorescence-Based System for Measurement of Transmembrane Potential Variations of Electrically and Mechanically Stimulated Cardiomyocytes

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Cardiomyocytes are electrically-active heart cells whose electrical properties vary with the local electrical and mechanical environment. Variations in myocytes electrical properties are known to play a role on abnormal rhythms.

The purpose of the project is to design a fluorescence-based photodetection system for measurement of transmembrane potential variations by combined electrical and mechanical stimulations in post-culture cardiomyocytes.

The isolated cardiomyocytes are seeded on a 10mm x 10mm x 0.127mm silicon sheet held by a pair of pliers, coupled to a stretcher apparatus made of two linear guide systems and two computer controlled linear stepper motors. The cells are kept in bubbled Tyrode solution and are electrically stimulated during the 10 minutes staining by the voltage-sensitive dye di-8-Anepps (Invitrogen) at 5 μmol/L concentration. Field electrical stimulation is done by a pair of parallel carbon electrodes with grounded anode. The cathode voltage is supplied by a bipolar isolation amplifier circuit whose input is a set of pulses from the digital-to-analog converter of a National Instruments card (NI USB-6221). The light source is a green LED array (wavelength = 523nm, NTE Electronics Inc.), with intensity controlled by a Darlington array receiving TTL signals. The emitted fluorescence is filtered ($\lambda > 610$nm), converted to voltage with a fast photodiode (S1226-5BK, Hamamatsu), and amplified by an instrumentation amplifier (AD524ADZ, Analog Digital Inc). The voltage is then digitized with a National Instruments card, filtered and saved for post-experiment analysis.

Each sub-system has been successfully validated. Testing the whole system with cardiac-derived HL1 cells allowed final improvement on the signal-to-noise ratio and optimization of excitation intensity. This ready to use bioinstrument will play a key role in further studies on cultured cardiomyocytes.

1747-Pos

In Vitro Cardiac Safety Profiling of a Novel Benzyl-Ethylamine Compound

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When developing novel compounds for any clinical indication, the possibility of untoward cardiovascular effects must be addressed. To evaluate the cardiac electrophysiologic effects of A-674563 ((S)-1-benzyl-2-[5-(3-methyl-1H-indazol-5-yl)-pyridin-3-yloxy]-ethylamine), a novel benzyl-ethylamine, we profiled the compound in a series of cellular and tissue assays. In canine Purkinje fibers (30 min exposure; 2 sec BCL), A-674563 elicited concentration dependent depolarization with shouldering of the terminal repolarization phase (20 μM) and increased abnormal automaticity (60 μM). In papillary muscles, concentration dependent depolarization was also seen, but the effect was much less potent; 60 μM induced shouldering of the terminal phase of repolarization. Contractility was assessed using percent changes in fractional shortening of sarcomere length (FS) in rabbit left ventricular myocytes. A-674563 reduced FS in a concentration dependent manner; 10% at 2 μM and 47% at 20 μM. Effects on ionic currents were further evaluated using heterologously expressed cardiac ion channel cell lines. A-674563 inhibited Cav1.2, expressed in HEK cells, with an IC_{50} of 20 μM, suggesting that the decreased contractility seen in native cells is due to L-type calcium channel block. The compound reduced Nav1.5 (HEK cells) by 50% at 20 μM and inhibited Kir2.1 (tSA201 cells) with an IC_{50} of 35 μM. Block of these channels would be expected to reduce the upstroke velocity, depolarize the cellular membrane and lead to abnormal automaticity as was seen in the tissue assays. Although A-674563 caused minimal prolongation of action potential duration in tissues up to 60 μM, it inhibited hERG (HEK cells) with an IC_{50} of 0.7 μM. This potent block was most likely offset by the concomitant block of multiple cardiac ion channels. In conclusion, A-674563 affects multiple cardiac ion channels to elicit depolarization and increased automaticity in native cardiac tissues.

1748-Pos

Stretch-Sensitivity of Stretch-Activated BK_{Ca} Channels in Post-Hatch Chick Ventricular Myocytes

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We have previously reported the electrophysiological properties of stretch-activated BK_{Ca} (SAKCA) channels cloned from cultured chick embryonic ventricular myocytes. However, the physiological role of SAKCA channels in the post-hatch heart *in situ* is not clear. We have investigated the effects on the SAKCA current of cell length changes, applied axially using a pair of carbon fibers attached to opposite ends of an isolated ventricular myocyte of a 2 week-old chick. Whole-cell currents were recorded using the patch-clamp technique, while the cells were either held at resting length, or stretched to cause a 10% increase in sarcomere length. Stretch did not affect whole-cell currents immediately after the stretch was applied. However, sustained stretch for 3 minutes significantly increased outward currents. This stretch-induced change was reversed by applying 10 nM Iberiotoxin, a specific BK_{Ca} channel blocker, or a Na^+/Ca^{2+} free environment. These results were reproduced in a computer simulation study, suggesting that stretch does not activate SAKCA channels directly, but does so in a secondary manner via a stretch-induced increase in the cytosolic Na^+ concentration followed by an increased Ca^{2+} influx.