myofilaments from TG hearts at both SL compared to NTG ones. There was an equal leftward shift of the force-pCa curves from both NTG and TG hearts when SL was increased to 2.3 μm. In addition, NEM-S1, a mimic of strongly bound, rigor cross-bridges, was not able to induce activation of TG myofilaments to the same extent as in the NTG controls. To determine whether isoform switching affects sarcomeric protein phosphorylation, we performed two-dimensional difference in gel electrophoresis (2D-DIGE) and Western blots using TM, TPM1κ, and Serine-283P specific antibodies. We observed an increase in the total phosphorylation of TPM1κ compared with that of α-TM. MLC2, TnI, and TnT phosphorylation was not significantly affected. Our results demonstrate that the increased cardiac expression of TPM1κ alters cardiac dynamics in a similar way to DCM-linked point mutations of TM.

1856-Pos

Distribution of Voltage Gated Sodium Channels in Rabbit Cardiomyocytes During Development

Cynthia Gershome[1,2], Eric Lin[1,2], Haruyo Kashihara[1,2], Glen F. Tibbits[1,2].
[1]Molecular Cardiac Physiology Group, Simon Fraser University, Burnaby, BC, Canada, [2]Cardiovascular Sciences, Child and Family Research Institute, Vancouver, BC, Canada.

We and others have postulated that in neonatal cardiomyocytes sodium currents may influence Ca^{2+} influx through reverse-mode NCX activity by inducing strong membrane depolarization and/or local sodium accumulation profoundly modify contractility. To study the role of voltage-gated sodium channels ($Na_V1.X$) and reverse mode NCX activity through development, the expression and distribution of $Na_V1.X$ was determined using Western blot analysis, immunocytochemistry, confocal microscopy and image analysis in cardiomyocytes isolated from 3 and 56 days old rabbits. Immunoblot analysis revealed a robust expression of the skeletal muscle isoform ($Na_v1.4$) in neonatal cardiomyocytes which decreased 6-fold by 56 days ($p<0.01$). The neuronal isotype $Na_v1.1$ was found to have comparatively low levels of expression throughout development and followed a similar pattern as that of Nav1.4. $Na_v1.1$ levels decreased by 9 fold in the 56 day cardiomyocyte ($p<0.01$). The cardiac isoform ($Na_v1.5$) expression was also robust in the neonatal and adult cardiomyocytes but the protein levels did not vary significantly throughout development by western blot analysis. The distribution of $Na_v1.4$ and $Na_v1.5$ was punctate in nature on the cell periphery in both 3 and 56 day cardiomyocytes. Both $Na_v1.4$ and $Na_v1.5$ co-localized with NCX in 3 and 56 day cardiomyocytes. In neonatal cardiomyocytes, $Na_v1.4$-NCX and $Na_v1.5$-NCX colocalization relationship remained the same, while in the adult, $Na_v1.5$-NCX colocalization decreased by 50% due to the increase in the separation distances between $Na_v1.5$ and NCX in the adult. Taken together, our results suggest that in the neonate heart, $Na_v1.4$ may dictate the role of NCX in regulating Ca^{2+} influx during contraction.

1857-Pos

Characterization of L-Type Ca Channel Currents ($I_{Ca,L}$) in Zebrafish Cardiomyocytes

Ping-Cheng Zhang[1], Xiao Ye Sheng[2], Leif Hove-Madsen[3], Glen F. Tibbits[1,2].
[1]Simon Fraser University, Burnaby, BC, Canada, [2]Child and Family Research Institute, Vancouver, BC, Canada, [3]Hospital de la Santa Creu i Sant Pau, Barcelona, Spain.

The zebrafish is an important model for the study of vertebrate cardiac development with a rich array of genetic mutations for functional interrogation. The similarity of the zebrafish cardiac action potential duration with that of humans further enhances the role of this model to study cardiac arrhythmias. Despite this, little is known about basic excitation-contraction coupling in the zebrafish heart. Single cardiomyocytes were isolated from the adult zebrafish heart by enzymatic perfusion of the cannulated ventricle. Using an amphotericin-perforated patch clamp in whole cell configuration, $I_{Ca,L}$ was characterized in the zebrafish cardiomyocytes at RT. Simultaneous recordings of the voltage dependence of $I_{Ca,L}$ amplitude and cell shortening showed a typical bell-shaped I-V relationship for $I_{Ca,L}$ with a maximum at 10 mV whereas cell shortening showed a monophasic increase with membrane depolarization, and reached a plateau at membrane potentials above 20 mV. $I_{Ca,L}$ was 53, 100, and 17% of maximum at −20, +10 and +40 mV while cell shortening was 62, 95, and 96% of maximum respectively, suggesting that $I_{Ca,L}$ is the major contributor to the activation of contraction at voltages below 10 mV whereas the contribution of reverse-mode NCX becomes increasingly more important at membrane potentials above 10 mV. The $T_{1/2}$ for the recovery of $I_{Ca,L}$ from inactivation was 96 ms and the $V_{1/2}$ for voltage-dependent inactivation was −27.6 mV. In conclusion, we demonstrate that: 1) healthy and viable myocytes can be obtained by enzymatic perfusion of the heart; 2) ventricular myocytes exhibited large $I_{Ca,L}$ density (>12 pA/pF) over a range of stimulation frequencies (0.5 to 3 Hz) and 3) a monophasically increasing contraction - voltage relationship which is in contrast to the bell-shaped relationship observed in human and other mammalian cardiomyocytes.

1858-Pos

Electrophysiological Determinants for Arrhythmogenesis Following Premature Stimulation In Murine Hearts

Rudolf M. Duehmke, **James D. Stefaniak**, Iman S. Gurung, Laila Guzadhur, Yanhui R. Zhang, Andrew A. Grace, Christopher L.H. Huang.
University of Cambridge, Cambridge, United Kingdom.

Background: Circus type re-entry is classically associated with reduced action potential (AP) conduction velocity through partially refractory tissue resulting in unidirectional conduction block. We assessed the extent to which premature extrasystolic APs under such conditions resulted in ventricular arrhythmogenesis in isolated Langendorff-perfused murine hearts.

Methods and Results: A novel programmed electrical stimulation (PES) protocol applied trains of 8 S1 stimuli at 100 ms intervals followed by extrasystolic S2 stimuli at successively decreasing S1S2 intervals. S2 stimulus strengths required to overcome refractoriness, reduce ventricular effective refractory period (VERP) and thereby elicit extrasystolic APs, increased with shortened S1S2 intervals, despite constant durations at 90% recovery (APD_{90}) of the preceding APs. Critical interval, CI, the difference APD_{90}-VERP, consequently increased with stimulus strength. The corresponding latencies and peak amplitudes of the extrasystolic APs consequently sharply increased and decreased respectively with CI thereby potentially replicating necessary conditions for re-entrant, circus-type, arrhythmia. The dependence of CI upon stimulus strength tended to consistent limiting values expected from approaches to absolute refractory periods. These values were greater in arrhythmogenic (mean CI 18.9 ± 0.55 ms, n=4) than in non-arrhythmogenic hearts (mean CI 15.1 ± 0.37, n=4; P=0.001, ANOVA), despite their statistically indistinguishable APD_{90} (arrhythmogenic hearts: 40.9 ± 2.23 ms, n=4 vs non-arrhythmogenic hearts: 36.5 ± 2.61ms, n=4; p>0.05, ANOVA) or VERP values (arrhythmogenic hearts: 22.5 ± 2.66 ms, n=4 vs non-arrhythmogenic hearts: 21.8 ± 2.53 ms, n=4; p>0.05, ANOVA).

Conclusions: These findings suggest existence of a specific CI (CI*) in turn corresponding to specific conditions of latency and action potential amplitude that would be sufficient to result in arrhythmogenesis.

1859-Pos

Effect of Sodium Homeostasis on Action Potential Duration Alternans in Cardiac Ventricular Cells

Leonid Livshitz, Yoram Rudy.
Washington University in St. Louis, St Louis, MO, USA.

Beat-to-beat alternation of action potential (AP) duration (alternans) is a precursor of fatal cardiac arrhythmias. The effect of the time course of intracellular Ca2+ transient on AP duration (APD) alternans was studied extensively, and the Na+/Ca2+ exchanger (INCX) was identified as a major coupling link between Ca2+ alternans and APD alternans. However, the role of Ca2+ -independent factors such as the Na+/K+ pump (INaK) in the coupling between the Ca2+ and AP subsystems has been overlooked.

We used computational models of AP and Ca2+ cycling in guinea-pig and canine myocytes to study effects of rate-dependent Na+ homeostasis on APD and the Ca2+ transient. We found that rate-dependent Na+ accumulation increases both the amplitude and frequency range of APD alternans in the guinea-pig, but decreases the amplitude of APD alternans in canine cells. The mechanism is as follows: in canine, Ca2+ and APD alternans are concordant (large Ca2+ is accompanied by long APD) and APD prolongation is due to inward INCX enhancement at a late phase of the AP. INaK enhancement by Na+ accumulation blunts the effect of INCX and decreases APD alternans amplitude. In the guinea pig, alternans are discordant (large Ca2+ transient with short APD) due to enhanced Ca2+ -dependent inactivation of L-type Ca2+ current and increased Ca2+-dependent slow delayed rectifier IKs at high Ca2+. Additional APD shortening by INaK increases the amplitude of the discordant alternans.

In conclusion, INaK enhancement due to Na+ accumulation decreases the amplitude of concordant APD-Ca2+ alternans and increases the amplitude of discordant APD-Ca2+ alternans. This mechanistic insight is relevant to arrhythmogenesis in heart failure where INCX is upregulated and INaK downregulated, amplifying APD alternans in larger mammals (canine) and possibly humans.