by 10.7% and 11.4%, respectively; CaT80 and CaT50 reduced by 6% and 5.3%, respectively). During maintained stretch, a gradual re-lengthening of APD and CaT duration was observed. After release of stretch, APD and CaT duration reverted to shorter values.

**Conclusion:** Living cardiac tissue slices offer a promising experimental model for the study of cardiac mechano-electric coupling. The methodology described can be refined (e.g. using a computer-controlled motorised stage to synchronise electrical and mechanical events, and by use of fiducial markers to track local tissue deformation rather than only input strain levels) and extended (e.g. exploring effects of stretch directionality, relative to prevailing cell orientation in a slice).

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559-Pos Board B339
Dual Spikes of Catecholamine Releases from Sympathetic Nerves in Rodent Heart Slices Following Hypoxia-Reperfusion as Recorded by a Novel Electrochemical Method

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Sympathetic nerve in heart is essential in cardiac physiology and diseases, because it releases catecholamines to regulate cardiac cells including atrial myocytes, atrial myocytes and vas through GPCRs. With a modified glass-insulated micro fiber carbon electrode (pegCFE), we record stimulus (depolarization or hypoxia)-signals via either amperometric current (I<sub>o</sub>) or fast cyclic voltammetry from the nerve terminals in rodent hearts, by a method termed cardiac Slice of Electrochemistry (cSEC). We found that, (1) cSEC signal I<sub>o</sub> is dependent on CF-foltage and extracellular Ca<sup>2+</sup>; (2) pharmacologically, I<sub>o</sub> is increased by Yohimbine, and decreased by forskolin; (3) electronic microscope detected dense core vesicles, tyrosine hydroxylase (TH) immunostaining and TH-GFP transgenic mice showed massive TH-signal in whole heart; (4) as determined by in-situ FCF, as well as microdialysis-based HPLC, NE and/or DHPG (a NE metabolic substance) were responsible for cSEC signals. These evidences establish that the evoked cSEC signals represent catecholamine releases from sympathetic nerves in heart slices. Using cSEC we discovered that hypoxia-reperfusion triggered dual spikes of catecholamine release at pH 7.4: first peak at 10s following hypoxia perfusion, second peak at 10s following normoxia perfusion. Finally, catecholamine release were reduced by 75% in ventricle slices from syt7(Ca<sup>2+</sup>-sensor)-KO versus WT mice, indicating cSEC may serve phenotyping of any sympathetic defects in cardiac disease animal models.

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Electronic Expression of IK1 in Human Induced Pluripotent Stem Cell Derived Cardiocytes Reveals Atrial vs Ventricular Specific Properties

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Cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs) provide a promising platform for understanding human cardiac pathol- og y and physiology. hiPSC-CMs differentiate into a mixed population of cells composed of ventricular, atrial, and nodal cell types; cellular phenotype is generally distinguished by morphology of the action potential (APs). However, in hiPSC-CMs a very small inward rectifying potassium channel (IK1) relative to native cardiac cells causes a depolarized membrane potential which contributes to inconsistent AP properties and misidentification of cellular phenotype. We used Electronic expression of IK1 via dynamic clamp to restore the AP and resting membrane potential back to a physiological levels. This allowed for improved discrimination of atrial and ventricular cells based on AP morphology. Using standard patch clamp techniques, we compared the inward sodium currents (INa) of cells with atrial and ventricular AP morphologies. INa current densities in cells with atrial like APs were larger than ventricular-like APs (at 30 mV, in pA/pF: -71.22 ± 6.96 (n = 5) vs. -46.25 ± 5.03 (n = 14); p < 0.05). Analysis of steady-state inactivation parameters of INa showed that cells with an atrial like AP had a more negative steady-state inactivation (V1/2: -78.14 ± 2.75 (n = 5) vs. -74.72 ± 0.8 (n = 14); p < 0.05). Cells with atrial APs had a larger Kr-1.5-like component at +50 mV than ventricular APs (in pA/pF: 3.71 ± 0.55 (n = 5) vs 1.00 ± 0.10 (n = 16); p <0.05) but similar peak currents: (6.89 ± 0.55 (n = 5) vs 6.85 ± 0.67 (n = 14)). IK1 current density was more than 3x smaller in cells with atrial-like APs at -120 mV (in pA/pF: -0.71 ± 0.08 (n = 5) vs. -3.45 ± 0.61 (n = 14); p < 0.05). These data suggest that differential AP morphology in hiPSC-CM has a similar electrophysiological basis to native cells.

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Normalization of Action Potential Properties in Human Induced Pluripotent Stem Cell Derived Cardiocytes by Electronic Expression of IK1

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Cardiac myocytes derived from human induced pluripotent stem cells (hiPSC- CMs) are a useful and renewable human myocyte model. Despite their promise, these cells have unexplored limitations when applied to action potential (AP) anal- ysis. APs occur spontaneously and are associated with variability due to a small/ missing inwardly rectifying potassium channel (IK1). We used electronic inward rectification clamp with dynamic clamp to express IK1, which significantly improved the physiolog- ical behavior of the AP and electrical profile of hiPSC-CMs. hiPSC-CMs have a negligible peak IK1 at ~120 mV (−0.81 ± 0.4 pA/pF) which results in depolarized resting membrane potentials (RMP) (−60.0 ± 1.7 mV; n = 17). “Electronic transfection” of IK1 into hiPSC-CMs results in re- establishing a physiological RMP (~84.0 ± 0.2 mV), increases the maximal upstroke velocity (from 82.1 ± 2.4 to 161.5 ± 11.5 mV/ms), reduces AP dura- tion, and increases the rate of repolarization (from 0.35 ± 0.03 to 1.1 ± 0.1 mV/ms, n = 17). Despite a detectable transient outward potassium current in hiPSC-CMs, “spike and dome” morphology is generally absent in spontaneously active cells; addition of electronic IK1 restored this morphology in 12 of 17 ventricular cells. It also restored the relationship between maximum up- stroke velocity and sodium current density. The stabilized membrane potential allowed systematic measurement of dynamic parameters. The rate dependence of the AP duration was measured in at different pacing rates from 4000 to 500 ms in 12 electronic IK1 expressing hiPSC-CMs and showed a classical monotonic restitution curve, with AP increasing with increased cycle length. By removing sodium channel inactivation, electronic expression of IK1 improves hiPSC-CMs utility in assess mechanisms involving sodium channels and phase 1 repolarization such as LQT3 and Brugada Syndrome.

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Activation of Ca<sup>2+</sup>-Dependent Cation Current by Fluid Shear Force in Atrial Myocytes

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Atrial myocytes are subjected to fluid shear force (FSF) during each contraction and relaxation. Ionic currents regulated by shear force and their molecular integ- rity in atrial myocytes have been ill-understood. We examined whether FSF activates specific current in atrial myocytes and underlying mechanisms for FSF-sensitive ionic current using whole-cell patch-clamp technique. A FSF of ~16 dyn/cm<sup>2</sup> was applied to entire single atrial myocyte using automated micro-puffing apparatus. A FSF-sensitive current (I<sub>FSF</sub>) was detected in lowly Ca<sup>2+</sup>-buffered (0.5 mM EGTA) atrial myocytes, but not in highly Ca<sup>2+</sup>-buffered (2.4 mM EGTA or 10 mM BAPTA) myocytes. The I<sub>FSF</sub> showed an outward rectification with a reversal potential of about -6 mV. The I<sub>FSF</sub> was inhibited by high concentrations (20-50 μM) of ryanodine and by replacement of external and internal cation with impermeable NMDG<sup>+</sup>, suggesting that I<sub>FSF</sub> is a Ca<sup>2+</sup>-release dependent cation current. Application of either transient receptor poten- tial melastatin subfamily 4 (TRPM4) inhibitor 9-phenanthrol or TRPM4- specific antibodies removed most of inward I<sub>FSF</sub> and ~80% of outward I<sub>FSF</sub>. However, stretch-activated cation channel blocker GoTx-Mtx-4 did not affect I<sub>FSF</sub>. Interestingly, I<sub>FSF</sub> was strongly inhibited by inositol 1,4,5-trisphosphate receptor (IP<sub>3</sub>R) blockers, 2-APB (2 μM) or xestospongin C. In addition, in atrial myocytes isolated from type 2 P<sub>IR</sub>(IP,R2) knockout mouse, I<sub>FSF</sub> was not detected, although 9-phenonanol-sensitive I<sub>FSF</sub> was recorded in wild-type myo- cytes. Co-immunostaining of TRPM4 and IP<sub>3</sub>R in rat atrial myocytes revealed peripheral localization of these proteins with some co-localizations. These results suggest that fluid shear stimuli may activate TRPM4 channels in atrial myocytes via Ca<sup>2+</sup> releases triggered by the activation of nearby IP<sub>3</sub>R2.

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STIM1 Increases Ca<sup>2+</sup> Stores in the Sarcoplasmic Reticulum of Adult Feline Ventricular Myocytes

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STIM1 is a Sarcoplasmic Reticulum (SR) membrane resident protein implicated in sensing and maintaining SR Ca<sup>2+</sup> levels. The role of STIM1 in the regulation
of SR Ca²⁺ stores in the normal and diseased heart is not well described. Our data shows that STIM1 is present at low levels in adult normal cardiac myocytes, but expression levels increase after cardiac injury. Because STIM1 is involved in regulating intracellular Ca²⁺ homeostasis, we hypothesize that increased STIM1 expression after cardiac injury may be involved in the disturbed Ca²⁺ cycling within diseased cardiomyocytes. Using cultured adult ventricular feline myocytes, we found that adenoviral vector mediated overexpression of STIM1 induces cell death in 80% of myocytes versus only 5% in uninfected controls. We also showed that rested state contractions were minimally increased in un-paced STIM1 overexpressing myocytes, compared with control myocytes (3 fold increase), suggesting that STIM1 increases SR Ca²⁺ stores under conditions in which SR Ca²⁺ stores are usually depleted. STIM1 overexpressing myocytes had increased Ca²⁺ transient peaks (measured with fluo-4) as well as increased contractions. We are currently exploring how STIM1 modifies SR load and mechanisms by which STIM1 alters excitation-contraction coupling. Our findings show that STIM1 can increase SR Ca²⁺ loading and this could have effects on contractility and arrhythmias in disease.

564-Pos Board B344
Regional Heterogeneity of the Inwardly Rectifying Potassium Current in the Canine Heart
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Background: The inward rectifier potassium current, IK1, regulates the terminal phase of repolarization of the action potential, as well as the resting membrane potential. Regional variation in IK1 has been noted in the canine heart, but there have been no previous studies of IK1 in atrial or ventricular myocyte preparations. We examined the properties and functional contribution of IK1 in isolated myocytes from ventricular, atrial and Purkinje tissue.

Methods and Results: Action potentials (AP) were recorded from canine left ventricular midmyocardium, left atrial and Purkinje tissue. The terminal rate of repolarization of the AP (as assessed by the minimum dV/dt) in ventricle, but not in Purkinje, depended on changes in external K⁺ (K⁺-jo). Isolated ventricular myocytes had the greatest density of IK1 while atrial myocytes had the lowest. Furthermore, the outward component of IK1 showed that ventricular cells exhibited a prominent outward component and steep negative slope conductance, which was also enhanced in 10 mM K⁺-jo. In contrast, both Purkinje and atrial cells exhibited little outward IK1, even in the presence of 10 mM K⁺-jo, and both cell types showed more persistent current at positive potentials. Expression of Kir2.1 in the ventricle was 76.9-fold higher than that of atria and 5.8-fold higher than that of Purkinje, whereas the expression of Kir2.2 and Kir2.3 subunits was more evenly distributed in Purkinje and atria.

Conclusions: Kir2.1 and Kir2.3 subunit expression vary dramatically in regions of the canine heart, these variations in IK1 properties could potentially explain the differences in the AP rate of repolarization between heart regions in response to K⁺-jo changes.

565-Pos Board B345
Transient Outward K⁺ Current Underlies Heterogeneity of Action Potential Duration and Early Afterdepolarization from Right Ventricle in Transgenic Rabbit Model of Long QT Type 1
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Introduction: Long QT syndrome type 1 (LQT1) is a congenital disease lacking slowly activating K⁺ channel (IKs), associated with polymorphic VT (pVT) and sudden cardiac death. Tissue heterogeneity has been proposed as an important factor to trigger and maintain pVT. We investigated ionic mechanisms that underlie regional differences in the formation of early afterdepolarization (EAD) using transgenic rabbit model of LQT1.

Methods: The initiation of pVT under isoproterenol was mapped using optical mapping and myocytes isolated from RV and septum were studied using voltage clamp and confocal calcium imaging.

Results: Optical mapping of LQT1 hearts showed pVT preferentially originating from right ventricle (RV) (16 of 18 pVTs), which was changed after perfusing transgenic RV with 0.5 mM K⁺ (K⁺-jo). Isolated ventricular myocytes from RV were more susceptible to pharmacological block by terfenadine. To address the functional role of IK1 and IKs in equine hearts, we recorded action potentials from arterially-perfused wedge preparations from the right ventricular wall at different pacing rates. In the presence of 10 μM terfenadine a marked prolongation of the action potential duration (from 420 ms to 495 ms at 90% of repolarization (APD90) at a basic cycle length of 2000 ms) was observed.

Conclusions: The expression of Kir7.1 and Kir1.1 in equine hearts suggests as in humans they are important for cardiac repolarization and we have demonstrated a functional role for Kir1 in equine hearts. The slower activation of equine IK7.1/KCNE1 may be an adaption to long action potential duration at rest and the slower deactivation may lead to current accumulation during fast rates and thus be important for decreasing action potential duration at faster heart rates.

567-Pos Board B347
Overexpression of Adenylate Cyclase 8 (AC8) in Mice Increases Intrinsic Heart Rate (IHR) and Reduces Heart Rate Variability (HRV), and Detaches HR and HRV from Autonomic Modulation
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While changes in HRV have been ascribed to changes in neural autonomic input to the heart, recent studies have shown that beating rate variability also exists in isolated sinoatrial (SA) node tissue and SA node cells (SANC). Adenylate cyclases are constitutively expressed in SANC, where they ensure a high basal level of phosphorylation of pacemaking proteins even in the absence of autonomic receptor stimulation. AC8 is a Ca²⁺-calmodulin-activated AC which, when overexpressed in mice, leads to an increased rate and amount of Ca²⁺ cycling from the sarcoplasmic reticulum. We hypothesized that AC8 overexpression would affect IHR and HRV in vivo.

ECGs were recorded from wild type (WT) C57 and transgenic AC8 mice under light anesthesia before and after dual autonomic blockade with atropine and propranolol. Time- and frequency-domain parameters of HRV were measured. AC8 mice displayed significantly higher basal and intrinsic HRs, and significantly lower basal and intrinsic measures of HRV (total power 13% of WT basal; 21% of WT intrinsic). Dual autonomic blockade caused reductions in most HRV parameters in WT mice, but had no effect in AC8 mice. A shift in β coefficients from log-log plots of FFT-derived power spectra (basal: –1.82 in WT to –1.17 in AC8; intrinsic: –1.62 to –1.10) indicated more fractal-like behavior in the HR of AC8 mice.

In summary, genetic manipulation of a cAMP-generating mechanism intrinsic to SANC modifies autonomic HR and HRV, but also dissociates HR and HRV from autonomic control. Thus mechanisms of intrinsic SANC autonomic modulation, in isolation of and in concert with autonomic neural impulses, can regulate HR and HRV in vivo.