to block by 4-AP. Consistent with these two latter findings, *Kcne4*$^{-/-}$ mice were less susceptible to 4-AP-induced QTc prolongation (i.e., beyond their existing QTc prolongation) compared to age- and sex-matched *Kcne4*$^{+/+}$ littermates. We conclude that Kcne4 regulates Kv1.5 *in vivo* in mouse heart and that disruption of this regulation is a primary basis for ventricular repolarization defects in *Kcne4*$^{-/-}$ mice. In human heart, Kv1.5 expression is restricted to the atrial myocytes; future studies should also be aimed at elucidating a potential atrial role for Kv1.5-KCNE4 complexes.

618-Pos Board B373

Atrial and Ventricular Myocytes have Different Arrhythmogenic Profiles in Response to Oxidative Stress and Hypokalemia

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Introduction: Oxidative stress and hypokalemia are arrhythmogenic in both atria and ventricles. Here we contrasted the pro-arrhythmic differences and similarities between isolated atrial vs. ventricular myocytes in response to these stressors, and assessed the influence of fibroblast-myocyte coupling.

Methods: Isolated patch-clamped rabbit atrial and ventricular myocytes were exposed to oxidative stress (1 mmol/L H_2O_2) or hypokalemia (2.7 mmol/L K_o) to induce early and delayed afterdepolarizations (EADs or DADs) and triggered activity. Action potentials were recorded in the current clamp mode or in the dynamic clamp mode, allowing virtual fibroblasts to be coupled to the myocyte in order to evaluate the influence of myocyte-fibroblast coupling on EADs and DADs. The capacitance, conductance, resting potential, fibroblast-myocyte gap junction conductance, and number of virtual fibroblasts could be programmed at will.

Results: H_2O_2 or hypokalemia readily induced DADs and triggered activity in both ventricular and atrial myocytes. However, EADs, which were bradycardia-dependent, were observed only in ventricular myocytes, and not in atrial myocytes, even when additional pharmacologic stressors known to induce EADs were added (isoproterenol and Bay K) in combination with H_2O_2 or hypokalemia. However, EADs could be induced in atrial myocytes by coupling the myocyte to one or more virtual fibroblasts. EADs (and DADs) were also further potentiated by coupling ventricular myocytes to virtual fibroblasts.

Conclusions: Isolated ventricular and atrial myocytes both develop DADs and triggered activity in response to oxidative stress and hypokalemia. Whereas ventricular myocytes also readily develop EADs, atrial myocytes require additional factors to develop EADs. The dynamic clamp results suggest that myocyte-fibroblast coupling may be one such additional factor. These findings highlight the potential importance of myocyte-fibroblast coupling as a synergistic factor promoting arrhythmias in atrial and ventricular tissue.

619-Pos Board B374

Activation of Small Conductance Calcium-Activated Potassium Channels by Sarcoplasmic Reticulum Calcium Release Attenuates Delayed Afterdepolarizations in Ventricular Myocytes

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Small conductance calcium-activated potassium (SK) channels are upregulated in ventricular myocytes from human patients and animal models of heart failure (HF). However, their activation mechanism and function in ventricular myocytes remain elusive. We overexpressed SK2 channels in adult rat ventricular myocytes using adenovirus gene transfer to test the hypotheses that activation of SK channels in ventricular myocytes requires calcium release from sarcoplasmic reticulum (SR), and that upregulation of SK currents contributes to reducing triggered activity. Simultaneous voltage clamp and confocal calcium imaging experiments in SK2-overexpressing cells demonstrated that depolarizing voltage steps resulted in transient outward currents sensitive to the specific SK channel inhibitor apamin. SR calcium release induced by rapid application of 10 mM caffeine evoked repolarizing SK currents, whereas complete exhaustion of SR calcium stores eliminated SK currents in response to depolarizing voltage steps, despite intact calcium influx through L-type calcium channels. Furthermore, apamin-sensitive SK currents were activated by pro-arrhythmic global spontaneous SR calcium release events (calcium waves, SCWs). Current-clamp experiments demonstrated that SK overexpression reduced the amplitude of delayed afterdepolarizations (DADs) resulting from SCWs and shortened action potential duration (APD). Immunolocalization studies revealed that overexpressed SK channels were distributed both at external sarcolemmal membranes and along the Z-lines, resembling the distribution of endogenous SK channels. In summary, SR calcium release is both necessary and sufficient for the activation of SK channels in rat ventricular myocytes. SK currents contribute to repolarization during action potentials and attenuate DADs driven by SCWs. Thus, SK upregulation in HF may have an anti-arrhythmic effect by shortening APD and reducing triggered activity.

620-Pos Board B375

Action Potential Conduction Velocity is Increased by Raised Intracellular Camp in the Intact Rat Heart via a CaMKII Mediated Pathway

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This study examines the changes in ventricular conduction (CV) and action potential (AP) shape in isolated rat hearts during prolonged exposure to raised intracellular cAMP. Adult male Wistar rats (300-500g) were euthanized and hearts removed and Langendorff perfused with modified Tyrode's solution at 37^{o}C. CV was recorded using custom electrodes placed on the epicardial surface of the left ventricle, which was paced continuously at 8HZ. The delay between the AP wavefront reaching two sequential sets of recording electrodes was used to calculate the CV. AP recordings were made using a voltage sensitive dye, di-4-ANEPPs, via a fiber optic light guide system (3mm diameter). Under control conditions the average CV was 54.9 ± 3.3 cm/s. Treatment with a combination of forskolin and IBMX (fsk+IBMX) increased CV by 7.1 ± 1.7% ($p<0.01$ n=7). AP duration at 75% (APD75) was also prolonged by 57.1 ± 2.5% and AP amplitude (APA) increased by 8.8 ± 2.0%. Pre-treatment of the heart with the Protein kinase A (PKA) inhibitor, H-89 (3μM), reduced APD75 response in fsk+IBMX to 30.2 ± 5.1% ($p<0.01$ n=7) but did not affect the CV response (increased by 9.2 ± 2.3%). APA changes were unaffected (9.4 ± 2.6%). Treatment with the CaMKII inhibitor, KN93 (5μM), abolished the CV response to fsk+IBMX (+1.25 ± 1.3%, $P<0.01$, n=7). APD75 prolongation was also reduced in the presence of KN93 (APD75 137.1 ± 6.6%), the APA response was unchanged (increased by 7.5 ± 2.5%). These results suggest that the cAMP-induced increase in CV is mediated by CaMKII, not PKA. Block of the CV response in KN93 was observed independent of changes in APA, therefore the CV response to cAMP does not appear to be mediated by AP upstroke changes and may be due to changes in intercellular resistance.

621-Pos Board B376

Action Potential Shape Differences Set Species-Dependent β-Adrenergic-Stimulation Response

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Background: The cardiac action potential (AP) shape is a species-dependent feature related to differences in ionic currents underlying repolarization. In guinea pigs (GP), dogs and humans, the AP is prolonged by a pronounced plateau phase. In canine and human, but not GP, myocytes, a spike-and-dome profile characterizes repolarization of specific regions within the heart and to a different extent according to heart rate. It is unclear whether the response to β-adrenergic stimulation is dictated by peculiarities of ion channels properties, or may results from differences in AP contour.

Aim: The aim of this project is to test whether the presence of the spike-and-dome in the AP contour is, by itself, able to modify the response of membrane current to β-adrenergic stimulation in a rate-dependent fashion.

Methods: We performed AP-clamp on GP myocytes with dog epicardial and endocardial AP waveforms to assess the contribution of the spike-and-dome in isoprenaline (ISO) sensitive current (I_{ISO}) at diastolic intervals (DI) of 1750ms and 300ms. We also performed dynamic clamp experiments on GP myocytes with a computational simulated canine transient outward current (I_{to}) to evaluate the ISO response on the AP duration (APD) in presence of an artificial spike-and-dome.

Results: We found that: 1) At DI1750, I_{ISO} is more inward with dog endocardial rather than epicardial waveform; this difference was not evidenced at DI300. 2) The injection of a simulated canine I_{to} is not sufficient by itself to affect the direction of APD changes during β-adrenergic stimulation.

Conclusions: The differences between dog and GP in setting β-adrenergic stimulation response are a species-dependent feature not only related to I_{to} and might be explained as a more complex mechanism involving AP shape and a diverse contribution of Ca^{2+} and K^+ channels during the AP.