Basal cAMP/Pka/Ca\^{2+} Signaling is Linked to Action Potential (AP) Rhythmicty of Sinoatrial Nodal Cells (SANC) as well as to their Firing Rate

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Compared to freshly isolated SANC (f-SANC), the spontaneous AP firing rate at 34 ± 0.5°C of cultured adult rabbit SANC (c-SANC) is reduced by 50% (from 2.79Hz to 1.35Hz), due to Gi protein suppression of basal cAMP/Pka/Ca\^{2+}-dependent signaling. Here we demonstrate that altered PKA-dependent modulation of basal intracellular Ca\^{2+} cycling also reduces AP rhythmicty of c-SANC.

The AP rhythmicty index (RI, fig.1A), i.e. the offset of the 3\textsuperscript{rd} peak from the autocorrelation function of AP records, or from power spectrum analysis is reduced in c- vs. f-SANC, and is associated with prolongation of spontaneous Local Ca\^{2+} Releases (LCR) period during diastolic depolarization and an increase in its coefficient of variation (0.199 ± 0.014 (n=41) for c-SANC vs. 0.122 ± 0.009 (n=32) for f-SANC, p<0.001). 

Acute b-adrenergic receptor stimulation by isoproterenol (ISO), phosphodiesterase inhibition by 3-isobutyl-1-methylxanthine (IBMX), or prolonged Gi suppression by pertussis toxin (PTX), which rescues impaired cAMP/Pka signaling in c-SANC, not only rescues the reduced AP firing rate, but also restores normal variability of LCR period and restores the rhythmicty of AP firing to the f-SANC level (fig.1B).

Regional Variations of the Effects of Acetylcholine in the Canine Heart

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Acetylcholine (ACH) release slows heart rate and atrioventricular conduction by stimulation of an inward rectifying current (IK,ACh) in atrial tissue. The effect of ACh on ventricular function is still debated. We compared the effect of ACh on APs in canine atria, Purkinje and venricular tissue as well as on ionic currents in isolated cells. Action potentials were recorded from endo- or epicardial slices, Purkinje fibers, or atrial preparations. Whole-cell currents were recorded under voltage clamp conditions and unloaded cell shortening.

In atrial tissue, application of ACh elicited a decrease in APD\textsubscript{90} in failing cells (p < 0.0001-0.05, n = 6-7 myocytes, ANOVA, Tukeys post-test).

The findings show that cells isolated from the monocrotaline treated RV required less current to cause a decrease in APD, which is consistent with an increased membrane resistance. Previous studies have reported a decreased expression of potassium channels in monocrotaline-treated myocytes, which may be a contributing factor responsible for the results observed.

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Mechanisms of Steeper APD Restitution in Rat Failing Right Ventricular Myocytes

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Pulmonary artery hypertension (PAH) causes the right ventricle (RV) to become hypertrophied and fail. During this process the RV undergoes electrophysiological remodeling with resultant changes in action potential duration (APD). This study evaluated changes in APD restitution in a rodent model of PAH.

Male Wistar rats were given a single i.p. injection of monocrotaline (60 mg/kg) or an equivalent volume of saline. When clinical symptoms of heart failure became apparent (3-4 weeks later) animals were euthanized and the hearts excised. RV myocytes were enzymatically isolated and used for a number of electrophysiological measurements. These included: measurements of APD at pacing rates between 1 and 9 Hz; an action potential (AP) clamp to impose the AP recorded at 1 Hz at pacing rates of 1, 2, 5 and 7 Hz; and measurements of APD during application of increased negative current pulses between 1 and 500 pA in amplitude at 1 and 5 Hz.

Myocytes from the failing right ventricle had a significantly longer APD and steeper APD restitution curve compared to sham cells. Despite this greater APD shortening, under action potential clamp, compensation currents in failing myocytes were smaller in amplitude as pacing frequency increased. Consistent with this observation, injection of negative current caused a greater decrease in APD\textsubscript{90} in failing cells (p = 0.0001-0.05, n = 6-7 myocytes, ANOVA, Tukeys post-test).

Changes of Axial Resistance following Mechanical Strain Prevail Over Stretch-Activated Currents in the Modulation of Conduction Velocity in Cardiac Cell Strands

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Tissue deformation and stretch-activated currents (I\textsubscript{SK}) exert a feedback on cardiac electrical function (mechano-electrical feedback). The effects of stretch on conduction velocity (CV) and its modulation by I\textsubscript{SK} are still debated. We investigated the dependence of CV on passive tissue deformation and its modulation by I\textsubscript{SK} in cultured cardiomyocyte strands and simulation studies.

Strands of neonatal rat ventricular myocytes were cultured on deformable substrates. CV was measured optically under control conditions, upon 10% shortening and 10% lengthening. Simulations were conducted in fibers of ten Tusscher et al. model cells. A quadratic dependence of myoplasmic resistance on cell length was incorporated and gap junctional resistance (equal to myoplasmic resistance under control conditions) was assumed to be unaffected.

I\textsubscript{SK} was implemented as a constitutively active non-specific monovalent cation current with a nonlinear dependence on deformation.

In cultured strands, CV decreased by 3.0% upon 10% shortening and increased by 3.9% upon 10% lengthening (n=25). In simulated fibers without I\textsubscript{SK}, CV decreased by 5.1% and increased by 4.2% upon 10% shortening and 10% lengthening, respectively, in agreement with the experiments. When I\textsubscript{SK} was incorporated at previously reported levels, it caused a slight resting membrane depolarization by ~1 mV in undeformed fibers, but no major alteration of the CV behavior (4.8% decrease at 10% shortening; 3.9% increase at 10%
lengthening). At large I_{Sac} levels causing substantial membrane depolarization (≥5 mV) and inactivation of the Na⁺ current, the dependence of CV on tissue deformation was blunted or even inverted, with lengthening causing conduction slowing.

Thus, during length changes of ±10%, axial tissue resistance and I_{Sac} modulate conduction in opposite directions. However, at physiological I_{Sac} levels, CV is primarily determined by axial tissue resistance.

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Resolution of Hypo-Osmotic Stress in Isolated Mouse Ventricular Myocytes Leads to Detubulation
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It has been recently shown that various stress-inducing manipulations in isolated ventricular cardiac myocytes may lead to significant remodeling of t-tubules. Osmotic stress is one of the most common complications in various experimental and clinical settings, and therefore, this study was designed to test a hypothesis that osmotic challenge may affect the integrity of t-tubules. T-tubular remodeling in mouse ventricular myocytes in response to various osmotic challenges was studied using two approaches: (1) electrophysiologically, by measuring membrane capacitance and I_{K1} tail currents originating from K⁺ accumulation in tubules, and (2) using confocal microscopy of fluorescent dextran traps in vesiculated t-tubules. In particular, application and removal of 0.6 T (60% of NaCl) hypo-osmotic solution to myocytes led to ~30% reduction in membrane capacitance, ~3-fold reduction in the amplitude of I_{K1} tail current and ~2-fold reduction in so-called I_{K1} "inactivation" (due to depletion of t-tubular K⁺) at negative membrane potentials – all being consistent with strong detubulation. Importantly, if confocal imaging experiments showed that extracellularly applied dextran traps become trapped inside the myocytes only upon removal of hypo-osmotic solutions (i.e. during shrinking phase) but not during initial swelling period. In light of these data some relevant previous studies, including those on EC coupling phenomena during hypo-osmotic stress, may need to be reinterpreted and the experimental design of future experiments should take into account the novel findings.

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Fluid Pressure-Activated Non-Selective Cation Current and CT Current in Rat Atrial Myocytes
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When intact atrial muscle is exposed to turbulant flow or high fluid pressure during valve diseases, it produces arrhythmias. Here we characterized a novel fluid pressure (FP)-gated ionic current (IPFP) in single rat atrial myocytes using a microperfusion method. The application of FP with a normal bath solution elicited a transient inward current (~1 pA/pF at 80 mV). The magnitude of IPFP was increased in a pressure-dependent manner. The removal of extracellular Ca²⁺ largely enhanced the IPFP and eliminated the current adaptation. Under physiological ionic gradients, the IPFP displayed an inwardly- and outwardly-rectifying current-voltage relationship with a reversal potential (E_rev) of approximately ~52 mV. The Cl⁻ channel blockers, DIDS and 9-AC, suppressed inward and outward IPFP by about 50% and 70-80%, respectively. In symmetrical Cl⁻ solutions, the E_rev was shifted rightward (~18 mV) and the outwardly rectifying IPFP was attenuated. In the symmetrical Cl⁻ conditions, removal of extracellular Na⁺ largely reduced inward IPFP, and produced a left shift of E_rev (~64 mV). In addition, the elimination of internal K⁺ shifted E_rev to ~+4.6 mV and decreased outward IPFP. Although low concentrations of extracellular Ca²⁺ blocked IPFP with a negative shift of E_rev, high concentrations of extracellular Ca²⁺ produced a right shift of E_rev. Gadolinium ion (Gd³⁺), the stretch-activated channel blocker, partially blocked the inward IPFP. In current-clamped cells, FP of the same magnitude elicited spontaneous membrane depolarization with repetitive action potentials and prolonged action potential durations. These results indicate that IPFP may activate an outwardly rectifying Cl⁻ channel and a Gd³⁺ - and Ca²⁺-sensitive non-selective cation channel that carries Na⁺, K⁺, and Ca²⁺.

1459-Pos Board B351
Cardiac Na/K-ATPase and Na/K-ACTPase Function is Altered in Ankyrin B Heterozygous Mice
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Ankyrin-B (AnkB) is a multivalent "adapter" protein that targets select membrane proteins to the cytoskeleton. Loss-of-function mutations in AnkB may cause ventricular arrhythmias and sudden cardiac death in humans. Direct interaction with AnkB is required for the membrane targeting and stability of Na/Ca exchanger (NCX) and Na/K-ATPase (NKA), key regulators of cardiac contractility and arhythmogenesis. However, it is currently unknown how AnkB modulates NCX and NKA function. To investigate this, we used AnkB heterozygous mice (AnkB⁺⁻) and their wild-type (WT) littermates. Cardiac myocytes from AnkB⁺⁻ mice show reduced expression (by ~20%) and altered localization of both NCX and NKA. In agreement with the lower protein level, we found slower decay of the caffeine-induced Ca transient (τ=7.4 ± 0.8 sec vs. 5.2 ± 0.6 sec) and reduced maximum rate of NKA-mediated Na extrusion (5.0 ± 0.5 vs. 6.4 ± 0.4 mM/min) in intact myocytes from AnkB⁺⁻ mice vs. WT. Thus, NCX and NKA transport function are reduced in AnkB⁺⁻/WT mice. We also measured the voltage-dependence of the currents carried by NCX (I_{NCX}) and NKA (I_{NKA}) using whole-cell voltage-clamp. I_{NCX} and I_{NKA} were recorded during descending voltage ramps, as Cd-sensitive and K-activated currents, respectively. I_{NCX} reflected the lower NCX expression in AnkB⁺⁻ myocytes, with no difference in the voltage-dependence vs. WT. In contrast, I_{NKA} had a significantly (p<0.001) steeper voltage-dependence in AnkB⁺⁻/WT myocytes. Thus, at ~80 mV, close to the resting membrane potential, I_{NKA} was reduced by ~53% in AnkB⁺⁻/WT mice, whereas at ~30 mV, close to the peak of the action potential, AnkB⁺⁻/WT was elevated by ~18% vs. WT. Thus, in addition to NKA protein expression, AnkB also directly modulates NKA function in cardiac myocytes, by reducing the voltage-dependent I_{NKA} inactivation. This could significantly affect myocyte [Na⁺] and [Ca²⁺] regulation.

1460-Pos Board B352
Electrophysiologic Effects of Azithromycin in Cardiomyocytes
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The widely-used macrolide antibiotic azithromycin (AZ) increases risk of cardiovascular and sudden cardiac death. Case reports indicate that AZ can cause polymorphic ventricular tachycardia in the absence and presence of QT prolongation, implying a novel proarrhythmic syndrome. We investigated the electrophysiologic effects of AZ in vivo and in vitro using mice, cardiomyocytes, and heterologously-expressed human ion channels. After implanting an ECG telemetry, conscious adult mice received intraperitoneal injection of AZ (50 mg/kg, followed in 60 min by 100 mg/kg; n=7). With both doses of AZ, heart rate declined (from 685 ± 24 to 489 ± 20 and 481 ± 21 bpm, for baseline, 50 and 100 mg/kg, respectively [mean ± SEM]; P<0.001). In addition, AZ increased the PR interval (32.7 ± 0.9 ms to 39.4 ± 0.7 and 39.8 ± 0.9 ms, respectively; P<0.001), QRS interval (10.2 ± 0.4 ms to 12.5 ± 0.4 and 13.3 ± 0.5 ms, respectively; P<0.001), and QT interval (37.4 ± 4 ms to 48.0 ± 5 and 51.2 ± 4 ms, respectively; P<0.01). In spontaneously-beating HL-1 cardiomyocytes, AZ (100 μM) significantly slowed heart beat rate (from 215 ± 7 to 180 ± 7 bpm; n=14; P<0.01), while increasing action potential rise time (23.0 ± 2.8 ms; P<0.01) and duration (at 90% repolarization, 118.3 ± 8 ms to 137.8 ± 8.7 ms; P<0.01). In HEK cells stably expressing SCN5A, AZ reduced Na⁺ currents (IC₅₀ 110 ± 3 μM; n=14), while similar results were obtained using mouse ventricular myocytes (IC₅₀ 117 ± 4 μM; n=6). In addition, AZ suppressed K⁺ currents recorded from HEK cells expressing hERG (IC₅₀ 219 ± 21 μM; n=5) and CHO cells expressing KCNQ1 and KCNE1 (IC₅₀ 184 ± 12 μM; n=6), as well as L-type Ca²⁺ current in rabbit ventricular myocytes (IC₅₀ 67 ± 4 μM; n=5). We conclude that azithromycin blocks multiple cardiac ion channels to prolong the PR, QRS, and QT interval in vivo, at concentrations achievable within the heart based on intracellular drug accumulation. These effects likely contribute to its novel proarrhythmic effect in humans.

1461-Pos Board B353
Enhancement of Antioxidant Defense Preserves RyR2 Function of Hyperglycemic Cardiomyocytes via Regulation of Both Intracellular Zn²⁺ and Ca²⁺ Homeostasis
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Zinc exists in biological system and resting intracellular level of free Zn²⁺ ([Zn²⁺]i) can be greatly increased by thiol-reactive oxidants or high glucose and contributes to oxidant-induced alterations in EC-coupling although in cardiomyocytes. Since [Zn²⁺]i, is altering function of numerous cellular proteins, its mobilization by reactive oxygen species in diabetic heart can be likely to cause significant effects. Therefore, we aimed to investigate the role of antioxidant-defence system in preserving of cardiac ryanodine receptor function.