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Optical Mapping Of VF In Isolated Swine Hearts With Scars

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Ventricular fibrillation (VF) is the main cause of sudden cardiac death. We hypothesized that VF induced by large scars in an isolated porcine heart model could aid the understanding of VF in human hearts associated with structural disease. The explanted hearts were perfused with blood and Tyrode solution at 37C, and optically imaged with a voltage-sensitive fluorescence dye (di4-ANEPPS excited at 530nm with 150W halogen lamp). The emitted signal was filtered (610nm) and recorded with high speed cameras (MiCAM02, BrainVison, Jp) at 0.7mm spatial resolution. No optical signals could be recorded from the core of chronic infarcts or RF lesions. A total of 10 hearts were used: 4 controls, 3 with lesions generated via RF ablation and 3 with chronic infarcts. We observed the propagation of the depolarization waves and analyzed the VF waveforms at the border zone (BZ) and normal myocardium. We analyzed the VF waves in the frequency domain by calculating the dominant frequency (DF) on select regions of interest using Matlab (Mathworks, Ca). Our results showed that DF is smaller at the BZ compared to healthy tissue. Referenced to the average DF in the control hearts (10.07+/-0.54 Hz), the DF was slightly smaller in healthy myocardium of infarct hearts (i.e., 8.9+/-0.71Hz) and significantly smaller at the border zone (i.e., 6.03 +/- 0.86Hz). In ablated hearts, mean DF in normal myocardium was 9.16+/-0.7Hz and 7.24+/-0.66Hz at BZ, respectively. We suggest that these differences are related to the heterogeneous restitution properties as well as the changes in tissue structure at the BZ. The BZ of chronic scars is comprised of a mixture of viable and necrotic fibers; whereas in the acute settings of RF lesions, inflammation and edema are present at the BZ without alteration of fiber directions.

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Negative Regulation of LQT2-Associated Kv11.1 Mutant Channels by Alpha(1A)-Adrenoceptors in Mammalian Cell Line

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Mutations in KCNH2 gene underlie type 2 of the congenital long-QT syndrome (LQT2), in which rapid component of I_K (I_{Kr}) is malfunctional and startled auditory stimuli are specific symptomatic trigger. The latter suggests fast arrhythmogenic mechanism. Therefore, we investigated acute alpha$_{(1A)}$- and cAMP-related beta-adrenergic modulation of I_{Kr} in HL-1 cardiomyocytes, wild type (wt-) and two LQT2-associated mutant Kv11.1 channels (Kv11.1-Y43D and Kv11.1-K595E) reconstituted in Chinese Hamster Ovary (CHO) cell line.

I_{Kr} and Kv11.1 currents were recorded through whole-cell patch-clamp technique and confocal microscopy of HL-1 cardiomyocytes transfected with GFP-tagged pleckstrin homology domain of phospholipase C-delta$_{(1)}$, visualized the fluctuations of membrane PIP_2 content.

In HL-1 cardiomyocytes expressing human alpha$_{(1A)}$-adrenoceptor, superfusion with 30 micromol/l phenylephrine significantly reduced I_{Kr} amplitude, shifted current activation to more positive potentials and accelerated kinetics of deactivation. Confocal images demonstrated decline of PIP_2 concentration during phenylephrine exposure. Stimulation of beta$_{(1)}$- and beta$_{(2)}$-adrenoceptor downstream enzyme adenylyl cyclase by 5 micromol/l forskolin shifted I_{Kr} activation to more negative potentials but did not significantly altered tail current amplitude. In parallel, alpha$_{(1A)}$-adrenoceptor activation downregulated reconstituted wt-Kv11.1 channels but forskolin (5 micromol/l) produced little effects. Expressed alone, Y43D-Kv11.1 or K595E-Kv11.1 channel had no measurable function. However, co-expression of wt-Kv11.1 and each mutant protein evoked currents with loss-of-function alterations but identical to wt-Kv11.1 alpha$_{(1A)}$- and forskolin-induced regulation.

Acute adrenergic regulation of at least two Kv11.1 mutant channels is preserved as in wt-Kv11.1 and native I_{Kr} and could have arrhythmogenic potential in some LQT2 cases.

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The Role Of Mineralocorticoid Receptors In The Adaptation Of Cardiac Myocytes To Pregnancy

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Aim: Pregnancy is characterized by a hypertrophic remodeling of the heart, but little is known about the role of hormonal regulation in this cardiac modification. Mineralocorticoid receptors (MRs) have been shown to mediate structural and functional remodeling of the heart in pathological conditions. Also, its agonists, glucocorticoids and mineralocorticoids, are significantly enhanced in pregnancy. Our aim is therefore to examine the possible role of MRs in cardiomyocyte adaptation during rat pregnancy. **Methods:** Pregnant rats were studied one day before parturition. One group of pregnant rats (Pcan) was treated with potassium canrenoate (20 mg/kg/day), a MRs antagonist, for the last seven days of pregnancy, and compared to normal pregnant rats (P). These groups were also compared to non-pregnant rats, treated (NPcan) or not treated (NP). M-mode echocardiography was performed for the whole heart study. Rapid video-imaging was used to record cell contractility at 0.5 Hz. Patch clamp technique was applied to study L-type calcium currents (ICa-L). **Results:** MR antagonism in Pcan induced a decrease of the systolic and diastolic dimensions of the heart, when compared to P. This result was corroborated by a lower cell volume in Pcan. Cell contractility was not modified in all groups, when glucose was the only energetic substrate. However, our results uncovered a modified responsiveness to energetic substrates lactate and pyruvate, naturally increased in the blood of P. Indeed, while cell contractility was raised in P in the presence of these substrates, this effect was not observed in Pcan. Interestingly, in Pcan, ICa-L tend to increase in the same energetic condition when compared to ICa-L with glucose only. **Conclusions:** Our data indicate that MRs are involved in the adaptation of cardiac myocytes to pregnancy at the structural, metabolic, as well as functional level.

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The Sialyltransferase, ST3Gal-IV, Modulates Cardiac Action Potential Waveforms And I_K

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Atrial arrhythmias can be caused by changes in atrial action potential (AP) waveform or conduction. The regulated activity of ion channels, including voltage-gated potassium (K_v) channel isoforms, is crucial to normal AP waveform. Each K_v channel isoform is uniquely glycosylated; glycans are typically terminated by sialic acid residues. Reports have shown sialic acids can modulate K_v channel gating through isoform-specific mechanisms. Here, we questioned whether regulated sialylation alters AP waveforms and voltage-gated K^+ currents (I_K) produced in the atrium. AP waveform parameters and two types of I_K, the transient outward, I_{to}, and the slowly inactivating, $I_{K,slow}$, were compared in atrial myocytes isolated from neonatal control versus ST3Gal-IV knockout animals. ST3Gal-IV is a sialyltransferase expressed at uniform levels throughout the heart and adds sialic acid residues to N- and O-linked glycans through α2-3 linkages. ECG recordings suggest that cardiac conduction/rhythm are altered in ST3Gal-IV$^{(-/-)}$ animals. AP duration (APD) was prolonged significantly in ST3Gal-IV$^{(-/-)}$ atrial myocytes compared to control APD. APD_{10}, APD_{50}, and APD_{90} values for ST3Gal-IV$^{(-/-)}$ myocytes were ~50-80% greater than control values ($p<0.004$). A reduction in K_v channel activity is one mechanism by which AP repolarization can be prolonged. To determine whether K_v channel activity is modulated by ST3Gal-IV expression, whole cell I_K from control versus ST3Gal-IV$^{(-/-)}$ atrial myocytes were measured and compared. The voltages of half-activation for I_{to} and $I_{K,slow}$ were shifted significantly by >10 mV to more depolarized potentials in ST3Gal-IV$^{(-/-)}$ myocytes compared to control ($p < 0.001$); a depolarizing shift in activation voltage will lead to fewer K_v channels active at a membrane potential, effectively reducing K_v channel activity. These data suggest that the regulated expression of a single sialyltransferase, ST3Gal-IV, can alter I_K, thus modulating the rate of atrial repolarization and potentially leading to arrhythmias.

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Ion Channel Toolbox for Cardiac Safety Evaluation

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The cardiac action potential is comprised of multiple ion channel currents acting in concert and these ion channels are important in cardiac safety liability assessment of potential drug candidates. Currently, hERG current screening is a critical part of the preclinical assessment of a drug candidate and is required before first in human (FIH) clinical studies. Through our ongoing efforts to provide efficient cardiac safety evaluation, while reducing animal usage, we have incorporated the use of several cellular ion channel whole-cell voltage clamp screens using heterologously expressed and native cardiac ion channels. With heterologously expressing cell lines, the use of planar patch technology (PatchXpress and QPatch) allows for moderate throughput by providing automated, simultaneous whole-cell voltage clamp recordings. In this study we highlight five cardiac ion channels; 3 heterologously expressed cardiac potassium channels (hERG [I_{Kr}], Kir2.1 [I_{K1}], KvLQT1/minK [I_{Ks}]) that contribute to the repolarization phase of the action potential and two additional cardiac ion

channels, whose primary contribution is to the upstroke and plateau of the action potential; Nav1.5 [I_{Na}] (heterologously expressed) and the native cardiac L-type Ca channel, Cav1.2 [I_{CaL}] (cardiac myocytes). The biophysical properties of these two cardiac ion channels have been extensively characterized and each ion channel assay has been pharmacologically validated with reference compounds. In conclusion, this approach is part of our continuing effort to move to a cellular based *in vitro* safety approach to provide mechanistic SAR for solving cardiac safety issues. To date, our ion channel toolbox includes: hERG, Kir2.1, KvLQT1/minK, Nav1.5 and the native cardiac L-type Ca channel (Cav1.2); to be used on an as needed basis for cardiac safety evaluation.

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Do hERG Enhancers and Blockers Compete?

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HERG (human ether a go-go related gene) encodes a cardiac potassium channel that has been linked to delayed repolarization. Due to the large vestibule of the hERG channel pore, many structurally dissimilar compounds are able to block the hERG channel. This, along with specific requirement of hERG data by regulatory authorities, has added to the difficulty of drug discovery. Recently, we have discovered a series of compounds (hACTs) that do not block hERG, but actually enhance hERG current. hACT-1({4-[4-(5-trifluoromethyl-1H-pyrazol-3-yl)-phenyl]-cyclohexyl}-acetic acid) enhanced hERG current by 50 % at 60 μM. In addition, hACT-1 caused concentration-dependent shortening of the action potential duration in canine Purkinje fibers and guinea pig atrial tissue. Preliminary studies suggest that binding of hACT-1 (60 μM) does not overlap sites of typical hERG blockers. hACT-1 did not displace radio labeled dofetilide. Also, in whole-cell voltage clamp studies, combination of hACT-1 with known hERG blockers (i.e., sotalol and terfenadine) suggest that the compounds are not competing for the same binding site. When applied simultaneously with a hERG blocker, the onset of hERG enhancement with hACT-1 occurs prior to block with either sotalol or terfenadine. Block with sotalol (150 μM) occurs at the same magnitude when used alone (42 %), or in combination with hACT-1 (44 %). Similarly, the enhancement of hERG current by hACT-1 is independent of sotalol block, just as the block of hERG current by sotalol is independent of hACT-1 current enhancement. These effects demonstrate that the binding site for enhanced hERG current is different than the binding site for block.

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A Novel *SCN5A* Mutation Associated With Brugada Type ECG And Intraventricular Conduction Defects

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Background: Mutations of SCN5A, gene encoding α-subunit of cardiac sodium channel, can cause mixed phenotypes of Brugada syndrome (BrS) and cardiac conduction diseases (CCDs).
Methods: We have identified a novel nucleotide change of *SCN5A* (4178T>G) which results in a nonsense mutation, L1393X, in a 36 year-old Caucasian male who presented with intraventricular conduction delays and BrS type ECG change. To study biophysical characteristics of L1393X-SCN5A, electrophysiological and immunostaining studies were performed using mammalian expression systems.
Results: While WT-SCN5A showed significant currents (93.3 ± 10.6 pA/pF; 1 μg plasmid), L1393X (5 μg) did not generate any significant currents in NIH-3T3 cells. The cells co-transfected with WT (0.5 μg) and L1393X (0.5 μg) showed approximately 50% current amplitudes compared to the WT (1 μg). Voltage-dependency of the steady-state activation and inactivation was not affected by the co-transfection of L1393X. Immunohistochemical stainings demonstrated that L1393X proteins were expressed in the plasma membranes.
Conclusion: Our study demonstrated that L1393X-SCN5A does not form functional channel proteins, which might account for the patient's mixed phenotypes of BrS and CCDs.

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Effects Of Silencing Synapse Associated Protein-97 On Cardiac Potassium Currents

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Introduction: Synapse associated protein-97 (SAP97) is a scaffolding protein expressed in cardiac myocytes. Previous studies have suggested that SAP97 interacts with and modifies properties of ion channels. We have investigated the functional effect of silencing SAP97 on major repolarizing currents in adult rat ventricular myocytes (ARVMs). **Methods:** SAP97 was silenced using a shRNA expressing adenovirus. The standard patch clamp technique was used to investigate the effects of this silencing on potassium currents in ARVMs. Control experiments were carried out in ARVMs infected with a GFP expressing adenovirus. **Results:** Western blot analysis showed that SAP97 was silenced in ARVM after 3 days in culture. In SAP97 silenced ARVMs, I_{K1} density was reduced by ~50% when measured at -100 mV (Holding potential (HP) = -50 mV). Average current density was -1.85 ± 0.3 pA/pF, n=12 as compared to -3.76 ± 0.5 pA/pF, n = 6 in control cells. Depolarization-activated (at +60 mV, HP = -70 mV) currents in the ARVM were fitted with a two-exponential function for analysis. Amplitude and kinetic analysis of the fits showed that there was a 30% decrease in the current density of the first component in SAP97 silenced ARVMs (9 ± 1.9 pA/pF, n=5) as compared to control (13.5 ± 0.9 pA/pF, n=4). SAP97 silencing however did not significantly change the kinetics of the first component. Time constants averaged 68 ± 12 msec and 1.1 ± 0.1 sec in control versus 121 ± 24 msec and 1.47 ± 0.21 sec in SAP97 silenced cells. Compared to the control cells, there was no change in the current density of the second component in SAP97 silenced cells. Current amplitude averaged 3.1 ± 0.4 pA/pF (n=5) and 3.7 ± 0.2 pA/pF (n=4), respectively for SAP97 silenced ARVMs and control. These results suggest that the silencing of SAP97 has differential effects on potassium currents in adult cardiac myocytes.

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Evidence And Functional Impact Of A New K+ Channel In Mouse Ventricular Fibroblasts

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In the heart, fibroblasts represent the major cell type. They contribute to the production of the extracellular matrix. Cardiac remodelling during pathological injury is associated with differenciation of fibroblasts into myofibroblasts. The aim of this study was to characterize at molecular and functional levels a new K conductance in these cells.
Among K channel transcripts which were screened by high-throughput real-time PCR, SUR2 and Kir6.1 mRNAs were found to be the most abundant. Western-blots showed that SUR2 and Kir6.1 protein expression levels increased with culture duration as fibroblasts differenciated into myofibroblasts. In the inside-out configuration of the patch-clamp technique, SUR2/Kir6.1 K channels were recorded and showed insensitivity to ATP, inhibition by glibenclamide and activation by pinacidil and UDP. These properties are similar to those reported by Yamada et al (1998) for the SUR2/Kir6.1 molecular signature. In the whole cell configuration, these channels gave rise to a macroscopic glibenclamide-sensitive current which was activated by pinacidil and which amplitude increased with culture duration. This current was also activated by the endogenous sphingolipid sphingosine-1-phosphate (S1P) at the nM concentration range. The activation of this current was found to stimulate cell proliferation and to decrease IL-6 secretion. All these functional effects occurred for culture duration greater than 5 days.
In conclusion this work shows for the first time the presence of a glibenclamide-sensitive current which appears during differenciation of fibroblasts into myofibroblasts. This SUR2/Kir6.1 current, which may be activated in pathological conditions where fibroblasts differentiate into myofibroblasts and where S1P level increases, may modulate cardiac ventricular function.

TRP Channels

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The TRP Domain Of TRPC3 Is Essential But Not Sufficient For Erythropoietin- Regulated Activation Of TRPC3

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TRPC3 and TRPC6 are nonselective calcium channels and two members of the canonical transient receptor potential (TRPC) subfamily expressed on human erythroblasts. Although they are 73% identical in their amino acids sequence, they respond differently to erythropoietin (Epo) stimulation. Epo stimulates a significantly greater increase in calcium influx through TRPC3 (236 ± 7% increase above baseline) compared to TRPC6 (74 ± 5% above baseline). TRPC6 also inhibits Epo-stimulated calcium influx in cells cotransfected