enhancing the mitochondrial ROS production with either antimycin or rotenone. Oscillations of IKATP could be either initiated or potentiated upon rapid, but not slow, transition to near-anoxia and they were closely paralleled by depolarization of delta Psi, indicative of a transient inability of the F1F0-ATPase to keep delta Psi. At elevated oxidative stress, rapid transition to near-anoxia caused a burst of H2DCF oxidation which correlated with an increased rate of IKATP activation. These results show that metabolic oscillations occur in cardiomyocytes at near-anoxia and that these oscillations are controlled by mitochondria through the rate of ATP hydrolysis which in turn depends on ROS production.

2736-Pos

A New Mathematical Cardiac Cell Model for the Elucidation of the Mechanisms of Reperfusion Arrhythmogenesis

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Reperfusion arrhythmias result from pathologies of cardiac myocyte physiology that develop when previously ischemic myocardium experiences a restoration of normal perfusion. The mechanisms of reperfusion arrhythmogenesis, which involve many components of a highly coupled nonlinear system, have been under investigation for many years. Despite these efforts, an effective therapy for the prevention of reperfusion arrhythmias has yet to be translated into routine clinical practice. Because of the highly complex nature of the problem, we have developed a cardiac cellular mathematical model tailored to the study of reperfusion arrhythmogenesis. This model allows more realistic simulations of ischemia and reperfusion than have been conducted previously, because it includes coupled intra- and extracellular pH regulation systems, as well as modification of the activity of ionic channels and exchangers secondary to changes in pH and the concentrations of ATP, ADP and other associated metabolites. We show that the model more closely reproduces experimental ischemia data than other existing models. Because of this, the model has strong promise for elucidating mechanisms of reperfusion arrhythmogenesis.

2737-Pos

Understanding Pro-Arrhythmic Effects of Drugs using Computational Models and Parameter Sensitivity Analysis

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Increased risk of ventricular arrhythmia is a dangerous side effect of many pharmacological agents. Often, drugs block the K^+ channel responsible for rapid delayed rectifier current (I_{Kr}), leading to delayed repolarization of action potentials, prolongation of the QT interval, and increased arrhythmia risk. Some drugs, however, block I_{Kr} potently but are nonetheless safe. In addition, the effects of a drug on action potential morphology depend not just on the channel that is blocked, but also on the other channels present in the cell, a concept known as "repolarization reserve." We have gained new insight into both phenomena through analysis of ventricular myocyte computational models with parameter randomization and multivariable regression. The most likely targets of a non-specific drug can be deduced from the relationship between action potential duration and drug concentration, if the data are compared to the parameter sensitivity analysis of an appropriate electrophysiological model. Simulations also provide insight into how the electrophysiological substrate of a ventricular myocyte affects the response of the cell to a drug that blocks I_{Kr}. Such a drug always prolongs action potential duration, but the effects can be either exacerbated or attenuated, depending on the characteristics of the other ion channels present. Specifically, simulations with a common human ventricular myocyte model suggest that the most important factors influencing the response to an I_{Kr}-blocking drug are: 1) the underlying density of I_{Kr}; 2) the density of slow delayed rectifier current I_{Ks}; 3) the voltage-dependence of I_{Kr} inactivation; 4) the density of L-type Ca^{2+} current, and 5) the kinetics of I_{Ks} activation. These simulations provide for a quantification of the important concept of repolarization reserve, and demonstrate how analysis of computational models can provide insight into the factors that influence adverse drug reactions.

2738-Pos

Properties of Time Domain Vs. Frequency Domain Methods used in Atrial Fibrillation

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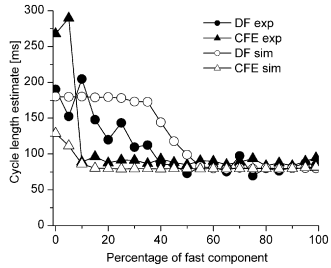
Complex Fractionated Electrogram (CFE) and Dominant Frequency (DF) are two methods commonly used to guide radio frequency ablation for treatment of atrial fibrillation (AF). CFE is based on the time domain and DF on the frequency domain.

We generated electrograms, composed of two components representing near- and far-field effects, with varying amplitude and white noise. Cycle lengths (CL) ranged from 80 to 180 ms. The electrograms were analyzed using time domain (CFE), and frequency domain (DF) methods, both in computer simulations and using the NavX system, routinely used in clinical practice to locate fast (<120ms) AF sources.

In computer simulations, DF approach estimated accurately CL of the fast (80 ms) and the slow (180 ms) signal and yielded 96 ms for equally combined signal. CFE method estimated CL of 129, 80 and 80 ms, respectively. When signals were fed into the NavX system via its hardware interface, DF yielded values of 190, 84 and 73 ms, respectively. CFE yielded 268, 85 and 95 ms, respectively.



The DF approach was more robust, since CFE tended to overdetect short CLs (see figure), thus unnecessarily prompting ablation more often than DF.

2739-Pos

Gender and Regional Differences in I_{cal} Distribution in Adult Rabbit Right Ventricle Influence Action Potential Duration and the Propensity for Eads in a Model of Long QT Syndrome Type 2

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Sex and apex-base differences in cardiac L-type calcium current (I_{CaL}) levels have been found to modulate vulnerability to arrhythmogenic early afterdepolarizations (EADs) in a drug-induced model of Long QT Syndrome Type 2 (LQTS2) in adult rabbit heart left ventricular epicardial myocytes. However, it is unknown whether similar gender and regional differences in I_{CaL} exist in the right ventricle. To further investigate the role of I_{CaL} as a determinant of EAD genesis, the apex-base distribution and biophysical properties of the calcium current in adult male and female right ventricles were assessed by the patch clamp technique and a modified Luo Rudy dynamic model of the cardiac action potential (AP). We found that I_{CaL} density measured at 0 mV was 48.2% higher in female (7.3 ± 1.2 pA/pF, n=6) compared to male base myocytes (3.8 ± 0.5, n=9, $p<0.008$). Analysis of regional differences in I_{CaL} in female right ventricle revealed 38.1% higher current density at the base (7.3 ± 1.2 pA/pF, n=6) compared to female apex myocytes (4.5 ± 0.5 pA/pF, n=8, $p<0.04$). There were no significant sex differences in I_{CaL} density in apex myocytes and no significant gender or regional differences in I_{CaL} activation and inactivation. Incorporation of I_{CaL} differences into the model showed that suppression of the rapid delayed rectifier potassium current to mimic LQTS2 resulted in increased AP duration and enhanced propensity for EADs in simulated female base myocytes. Taken together, these data demonstrate that sex and apex-base differences in right ventricle I_{CaL} correlate with the LQTS2-arrhythmia phenotype found in adult rabbit left ventricular epicardium and support the hypothesis that higher I_{CaL} underlies the propensity for EAD genesis.

2740-Pos

The Inter-Dependency of Local Myocardial Metabolism and Epicardial Electrical Activity during Acute Ischemia and Reperfusion

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Metabolic changes caused by the lack of adequate coronary flow lead to short and long term disturbances in local activation sequences. Our goal has been to study short term disturbances using parallel fluorescence imaging of epicardial NADH (fNADH) and transmembrane potential (TMP). METHODS: Experiments were conducted using Langendorff-perfused rat hearts while controlling the rate of flow to the left anterior descending coronary artery (LAD). Acute regional ischemia was induced by stopping flow to the LAD, followed by a period of low-flow reperfusion with subsequent full-flow reperfusion. Changes in local epicardial conduction velocities, as well as the incidence and dispersion of epicardial breakthroughs, were analyzed with the corresponding local changes of fNADH. With this approach, conduction velocities and reentrant activity could be correlated with changes in fNADH. RESULTS: Regional ischemia led to a reduction in Purkinje fiber activity within the ischemic zone. Approx 4 minutes after the initiation of ischemia, conduction velocities increased within regions with elevated fNADH. Afterward, conduction velocities in the ischemic zone declined and were lowest in the center, eventually falling to values below 20 cm/sec. Reductions in conduction velocity lagged behind

elevations of fNADH, both in time and space. During regional ischemia, occasional breakthroughs occurred, most of them along the boundary between ischemic and normoxic tissue. During low-flow reperfusion, the number of breakthroughs within the ischemic zone increased dramatically as well as the incidence of ventricular fibrillation (compared to ischemia and normal flow conditions). CONCLUSIONS: The inter-dependence of local activation patterns and local myocardial metabolism makes parallel imaging of fNADH and TMP an essential tool for understanding the mechanisms of arrhythmias caused by ischemia and reperfusion.

2741-Pos

The Transient Outward Current Ito Promotes Early After depolarizations

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The transient outward current (Ito) plays important roles in action potential (AP) morphology and arrhythmogenesis in cardiac diseases, such as ischemia and the Brugada syndrome. It is well accepted that early afterdepolarizations (EADs) occur under conditions of reduced repolarization reserve, which can result from either increased inward currents or reduced outward currents. Here we show the novel finding that Ito, an outward current, promotes EADs in rabbit ventricular myocytes, raising the question: how does an outward current promote EADs? To answer this question, we carried out experimental studies in isolated rabbit ventricular myocytes, theoretical analysis, and computer simulations. In myocyte experiments, exposure to 0.2-1 mM H2O2 at slow pacing rates induced EADs, which were eliminated by selectively blocking Ito with 2 mM 4-aminopyridine. Pre-treating myocytes with 4-aminopyridine prolonged AP, but likewise prevented H2O2-induced EADs. Voltage-clamp experiments showed that besides promoting late ICa,L and late INa, H2O2 also increased the maximum conductance, slowed the inactivation and accelerated the recovery from inactivation of Ito. When the cells were clamped with AP morphologies corresponding to the absence and presence of Ito, Ito significantly enhanced the Ca current, promoting its reactivation as the mechanism induced EADs. In a computer model of the rabbit ventricular AP, we also showed that the presence of Ito promoted EADs. The rate of Ito inactivation played a critical role: if too fast, no EADs occurred, and if too slow, AP duration became too short and no EADs occurred either. The underlying dynamical mechanisms were revealed by bifurcation theory of EADs previously developed by our group (Tran et al, Phys. Rev. Lett. 2009; 102:258103).

2742-Pos

Potassium Channels in Fetal Human Cardiomyocytes Compared to Rat and Rabbit

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Some side effects of medical drugs are caused by block of cardiac ion channels leading to cardiac arrhythmia. Because of different sets of ion channels in rat and rabbit, and adult and fetal humans, the conclusions on side effects are difficult to translate from species to species and even within one species. This study investigates the differences in potassium currents during the most vulnerable period of the development of the heart and compares the human fetal cardiomyocytes with rat and rabbit to understand the differences in drug effects on the heart's function between the species.

In rat we have used two time points for the study. Potassium channels at the embryonic day 11 (E11), the most vulnerable time point for the heart, are compared with E15, a much less vulnerable time point. E11 is also compared with E10 from rabbit and fetal human cardiomyocytes. The fetal human cardiomyocytes are from week 5 to 9, also in the risk period. We have studied the potassium currents IKr, IKs, and IK1 by the patch-clamp technique. We have also investigated the importance of the currents in generating action potentials.

2743-Pos

Action Potential Duration Modulation by Activation Sequence in Rat Vs. Pig Myocardium

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Dispersion of the cardiac action potential duration (APD) is known to influence the susceptibility of cardiac tissue to arrhythmias. Several experimental studies have revealed that APD can be modulated by the activation sequence. Our lab has recently shown strong correlations between APD and activation time (AT) in hearts from small rodents. However, a recent computational study indicated that the magnitude of such APD modulation may not be consistent across species. Therefore, the present study sought to compare experimentally APD modulation by activation sequence in rat and pig.

Optical imaging using the voltage-sensitive dye Di-4-ANEPPS was performed in Langendorff perfused rat hearts (n=4) and coronary perfused pig left ventricular slabs (n=5). The left ventricular mid-free wall was paced at 6Hz (rat) and 2Hz (pig), close to their intrinsic heart rate, and optical action potentials were acquired for 5s.

The mean APD near the pacing site (at 4ms AT) was 44.9 $\pm$ 9.4 ms in rat and 142.2 $\pm$ 11.3 ms in pig. A significant decrease of APD was revealed at larger AT in rat (37.2 $\pm$ 9.5 ms at 10ms AT, P0.05). Plotting APD as a function of AT revealed a linear correlation of APD with AT. Slope analysis revealed a decreasing trend in rats (mean slope $= -0.79 \pm 0.26$) whilst pigs showed no such modulation of APD (mean slope $= 0.29 \pm 0.22$) Heterogeneity, defined as the APD covariance over the whole field of view, was 0.10 in rat and 0.05 in pig ($P<0.05$).

In conclusion, APD can be strongly modulated by the activation sequence in hearts from small rodents whilst this modulation is absent in pig myocardium. This study emphasises the importance of APD heterogeneity induced by activation sequence and differences between species.

2744-Pos

Pressure Puff Induced Calcium Signals in Voltage Clamped Cardiomyocytes

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I_{Ca}-gated release of Ca^{2+} from the SR is the dominant mechanism mediating cardiac E-C coupling. On the other hand, in the absence of Ca^{2+} entry, supplemental Ca^{2+} release may be activated mechanically, either from the SR secondary to nitrosylation of RyRs or from mitochondria as a direct effect of puff-induced shear force. Here we have probed the puff-induced Ca^{2+} release in voltage-clamped cardiomyocytes, where the Ca^{2+}-indicator rhod-2 was targeted to mitochondria by: a) staining with the AM-form of the dye for 45 minutes, b) incubating without dye for 24-30 hours and c) dialyzing with dye-free internal solution through the voltage-clamp pipette for 20 minutes before initiating measurements. In such cells, which remained responsive for periods as long as 2 hours, we measured relatively slow (~1s) puff-induced deceases in fluorescence suggestive of mitochondrial Ca^{2+} release as previously found in non-dialyzed and permeabilized cells (Belmonte and Morad, 2008, J Physiol 586:1376). To clarify the $[Ca^{2+}]_i$- signaling under these conditions, our experimental paradigm included activation of I_{Ca} both at the beginning and end of a 2 second priming interval where the cell was exposed to control solution, 10 mM Caffeine or zero Na^+ (and high K^+). The caffeine-induced Ca^{2+} signal was biphasic with internal solution containing 0.2 or 14mM EGTA, generating I_{NaCa} only in 0.2 but not in 14mM EGTA during the rapid initial rise of Ca^{2+}, suggesting that the maintained component of the Ca^{2+} signal arises from a confined and most likely mitochondrial space, not detected by NCX. We conclude that patch clamped Rhod-2 loaded myocytes that were washed overnight and dialyzed for 1-2 hour with 14mM EGTA produced reliable mitochondrial Ca^{2+} signals supporting the finding that the PF-triggered Ca^{2+}-transients are caused by mitochondrial Ca^{2+} release.

2745-Pos

Regulation of the Transient Outward Potassium Current I_{tof} in Cardiac Hypertrophy by Sphingosine-1-Phosphate Signaling

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The fast transient outward potassium current (Ito,f), which is carried by voltage-gated Kv4.2 and Kv4.3 potassium channels and auxiliary subunit KChIP2, plays a critical role in early repolarization of the cardiac action potential. Ito,f and its gene products are strongly down-regulated in cardiac hypertrophy and disease, leading to altered excitation-contraction coupling and electrical activity as well as hypertrophy. Despite the importance of Ito,f in normal and diseased hearts, the regulation of Ito,f remains poorly understood. Studies have shown that the biologically active sphingolipid, sphingosine-1-phosphate (S1P), induces cardiac hypertrophy. In addition, the inflammatory pro-hypertrophic cytokine TNF-alpha, which decreases Ito,f, activates sphingosine kinase 1, the highly regulated enzyme that produces S1P. Therefore, we investigated the role of TNF-alpha and S1P signaling in mediating the down-regulation of Ito,f. In cultured neonatal myocytes, the TNF-alpha inhibitor etanercept attenuated reductions in Ito,f current density that were caused by the hypertrophic agonist phenylephrine (PE). Inhibition of sphingosine kinases by dimethylsphingosine prevented reductions in Ito,f that were caused by PE. Furthermore, application of S1P reduced Ito,f current density and caused hypertrophy. To interrogate the down-stream events involved in TNF-alpha/S1P- induced reductions in Ito,f, we focused on NF-kappaB since it is one of