demonstrate that Kif5b is required for the forward trafficking of newly synthesized Kv1.5 channel to the plasma membrane. This work has been extended to adult rat cardiomyocytes transfected with Kif5b constructs and wild-type and dominant negative Rab-type small GTPases. Results indicate that newly synthesized Kv1.5 traffics via a non-conventional pathway and on to the plasmalemma in a Kif5b-dependent process.

2731-Pos

The Cytopatch Instrument: the New Automated Patch Clamp Standard in a Comparative Study to the Manual Patch Clamp Technique Regarding the High Data Quality and Flexibility in Assay Design

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Manual patch clamp is known as the gold standard for investigating ion channel modulation. The high data quality is achieved at the expense of very low throughput, a low standardization feasibility and the need of an experienced operator. Here, it is shown that with the fully automated patch clamp platform CytoPatch Instrument the high data quality known from the manual patch clamp can be achieved, combined with a complete process and assay automation, resulting in the increased throughput needed for screening purposes. Based on the unique design of the Cytocentrics Chip with its dedicated micro fabricated glass pipette, the patch clamp process of the manual patch clamp is resembled. With the advanced microfluidic system various defined and precisely triggered perfusion protocols can be executed. This results in the same flexibility, giga seals, data quality and stability of recordings as it is known for manual patch clamp.It is shown that the CytoPatch Instrument can be used for electrophysiological characterisation of different ion channels. Dose-response relationships of typical hERG blocking compounds were generated using the CytoPatch Instrument. These are in excellent accordance with the data generated using the manual patch clamp technique. Furthermore, it is shown that the CytoPatch Instrument can be used for more advanced electrophysiological studies, e. g. the discrimination of different blocking mechanisms of compounds acting on the hERG ion channel. This study demonstrates that patch clamp automation with the CytoPatch Instrument can extend the standard screening process by more advanced studies. Furthermore, the CytoPatch Instrument is highly standardized and can be utilized in GLP studies.

2732-Pos

KCNH2 Channel Activators Increase I_{kr} in Hl-1 Cardiomyocytes and May Prevent the Occurrence of Torsades De Pointes in Long QT Syndrome

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Introduction: Several KCNH2 channel activators may provide a novel pharmacological approach for the treatment of long QT syndrome (LQTS). We therefore investigated the effects of the activators on I_{Kr} and action potential of cardiac myocytes.

Methods: We characterized the actions of three KCNH2 channel activators, mallotoxin (MTX), PD-118057 (PD), and NS1643 (NS) on I_{Kr} of HL-1 cardiomyocytes, using the whole cell patch clamp technique. With a mathematical model of human ventricular myocytes, we further evaluated the impact of activator-induced changes in I_{Kr} kinetics on the action potential configuration in normal and LQTS.

Results: The maximum tail currents of I_{Kr} were 23.4 ± 3.5 nA/pF with 10 μM MTX (n=12), 22.1 ± 2.7 nA/pF with 10 μM PD (n=13), and 23.3 ± 2.7 nA/pF with 10 μM NS (n=16), which were significantly greater than 12.8 ± 1.0 nA/pF in control (n=38). The half-maximal activation voltage was significantly shifted from -1.8 ± 2.7 (n=38) to -13.0 ± 2.3 (n=11), -8.3 ± 2.1 (n=13), and -14.7 ± 3.2 (n=14) mV by MTX, PD, and NS, respectively. Deactivation during the repolarization to −40 mV was significantly slowed by MTX, but not by PD or NS. The half-maximal inactivation voltage was significantly shifted from -6.6 ± 2.2 (n=28) to -29.9 ± 2.9 (n=15) mV by MTX, but not by PD, and NS. Simulation study showed that the activator-induced changes of I_{Kr} increased the amplitude of I_{Kr} during phase 2 of action potentials and consequently shortened the action potential duration by 19.7-23.6% in LQT1 and LQT3 models. A reduction of I_{Kr} in the LQT3 model evoked early afterdepolarization, which was abolished by the activator-induced enhancement of I_{Kr}.

Conclusion: KCNH2 channel activators, mallotoxin, PD-118057, and NS1643 increases I_{Kr} through distinct kinetic mechanisms and can be utilized for potential therapy of LQTS and torsades de pointes.

2733-Pos

Community Effect to the External Electrical Stimulation on Cardiomyocytes by using *On-Chip* MEA System

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In the cardiomyocyte network, network size and spatial arrangement of different cellular-type are important factors for reducing the fluctuation of the beating rhythms. To study the community effect on the cell network, experimental conditions need to control the community size and to construct the network in a stepwise manner. Therefore, we tried to develop a various size of cultivation chamber using the agarose micro-chamber system combined with the multi-electrode array (MEA) measurement system (we call it *On-Chip* MEA system). As first step for the cultivation of a single cell or small community (about 5-9 cell), we used agarose-gel as chamber material, which is one of a non cell-adhesive one. The agarose was spin-coated and its layer was fabricated to form the micro-chamber using 1480 nm photo-thermal etching. Then, cardiomyocytes were put in chamber with cell handling technique by micropipette. Next, to measure the extra-cellular signal and stimulate the cells noninvasively, we built up the MEA system with amplification and electrical stimulator. This system has highly gain (x 50k) capable of obtaining the field potential from single cell and 100 kHz of sampling rate (time-resolution: 10 micro seconds) enables us to capture the intercellular conduction of excitation. And then, it is able to control the stimulation at the multiple electrodes of 64 channels. Using these systems, we test the community effect about responsive band to pacing frequency. Cardiomyocytes purified from *mouse* embryonic hearts showed individual responsive band to pacing frequency, and band width were narrow. When cardiomyocytes formed community, responsive band were broaden dependent to number of cell. It indicates that community effect to external electrical stimulation depends on community size.

2734-Pos

Community Effect on Drug Sensitivity of Cardiomyocytes Controlled Spatial Patterns by using *On-Chip* MEA System

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In cardiomyocyte network, network size and spatial arrangement of different cellular-type are important factors for stabilization of beating rhythms. To study the community effect of the geometric factor and community size, we tried to develop cultivation chambers in a various size and geometric patterns using the agarose micro-processing technique combined with the multi-electrode array (MEA) measurement system (we call it *On-Chip* MEA system), and each of extra-cellular signal of mouse embryonic cardiomyocytes to tachyrhythmia inducing drug response was recorded simultaneously.

Firstly, to evaluate the effect of community size on the sensitivity to drug, we tried to build the square sheet type chambers having small, medium and large area (about 10^4, 10^5, and 10^6 um^2, respectively). Next, we tried to construct circuit type chambers with loop structure (circuit length of 2um, 8.2um) compared with sheet type geometry.

As a result, we observed different response to drug among community size and geometric pattern. These results imply that sensitivity to the drug depends on spatial patterns. In this meeting, difference of drug sensitive event (e.g. tachyrhthmia, cardiac arrest etc), we report in detail about quantitative parameters: Beating Rate (BR), Field Potential Duration (FPD), Short Term Variability (STV) of them etc, which will be help for understanding community effect.

2735-Pos

Mitochondrial Reactive Oxygen Species Control Metabolic Oscillations in Cardiomyocytes at Near-Anoxia

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Metabolic oscillations frequently occur under conditions simulating ischemia. As oxygen tension is a determinant of mitochondrial function and reactive oxygen species (ROS) production, we studied metabolic oscillations in single resting cardiomyocytes at near-anoxia ($pO2 < 0.1$ mm Hg) using on-chip picochambers. Activation of current through sarcolemmal KATP channels (IKATP), sensing the cytosolic ATP concentration, was measured simultaneously with either the mitochondrial membrane potential, delta Psi (TMRM fluorescence), or the cellular redox state (H2DCF fluorescence). Upon transition to near anoxia, activation of IKATP started with one or several current oscillations, which were time-correlated with oscillations of delta Psi and H2DCF oxidation. Metabolic oscillations persisted in cells treated with either cytoplasmic ROS scavengers or mitochondrial inhibitors of ROS production, and were stimulated when

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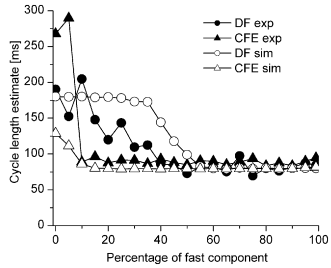
Complex Fractionated Electrogram (CFE) and Dominant Frequency (DF) are two methods commonly used to guide radio frequency ablation for treatment of atrial fibrillation (AF). CFE is based on the time domain and DF on the frequency domain.

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