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