them. Action potential onset occurred synchronously within the TAT network of NTG and TG cardiomyocytes. Whereas the repolarization time course was similar between the three recording sites in NTG or TG hearts (Figure A), overall repolarization was prolonged in TG cardiomyocytes (Figure B). Thus, electrical coupling between the surface and TAT membrane was maintained despite pronounced TAT membrane restructuring in this model of cardiac hypertrophy.

2726-Pos

Unraveling of a Novel Cation Current in Cardiac Myocytes using Fenamates

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Interest in non-selective channels has increased recently following the discovery of transient receptor potential (TRP) proteins, which underlie many of these channels. We used the whole-cell patch-clamp technique on isolated ventricular myocytes to investigate the effect of fenamates on membrane ion currents. With voltage-dependent and other ion channels inhibited, all cells that were challenged with either N-(p-amylcinnamoyl)anthranilic acid (ACA, $\geq$ 3μM), ONO-RS-082 ($\geq$ 100μM) or flufenamic acid ($\geq$ 100μM) responded with an increase in currents (induced current: -0.8 ± 0.06 pA/pF at -120 mV with 30 μM ACA; n=9). ACA was the most potent ($K_{0.5}$ =13 μM) of all drugs tested. The induced current reversed at $+43 \pm 2.2$ mV (n=9) and its inward but not outward component was suppressed in Na^+-free extracellular conditions (Na^+ replaced by $NMDG^+$). The current and its reversal potential (E_{rev}) were unaffected by lowering extracellular Cl^- concentration or by the removal of extracellular Ca^{2+} and Mg^{2+}. The current could not be induced by other non-fenamate anti-inflammatory drugs such as diclofenac, nor by non-fenamate phospholipase-A_2 inhibitors such as bromoenol lactone and bromophenacyl bromide. Muscarinic or α-adrenergic receptor activation or application of diacylglycerol failed to induce or enhance the current. The lack of effect of removing extracellular divalent cations and the fact that the induced current could be obtained in the presence of high intracellular Mg^{2+} indicated that the channel implicated is not TRPM7. Given our experimental conditions, where Na^+ is the only ion with an equilibrium potential close to the above E_{rev} values, it is very likely that Na^+ carries the novel current induced by fenamates.

2727-Pos

Reductions in Ventricular Ca^{2+} Current Occur Independently of Cardiac Remodelling in Transgenic Mice with Cardiac Specific Overexpression of the Human Type 1 Angiotensin II Receptor

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Transgenic mice with cardiac specific overexpression of the human type 1 angiotensin II receptor (AT1R mice) develop hypertrophy and decreased cardiac contractility. However, it is unclear whether altered contractility is attributable to hypertrophy or AT1R overexpression and whether this differs between sexes. Since L-type Ca^{2+} current (I_{CaL}) is crucial for cardiac contraction, we characterized the effects of AT1R overexpression on ventricular I_{CaL} in the presence (older mice: 6-month) or absence (younger mice: 50-day) of cardiac hypertrophy in both male (M) and female (F) mice. Voltage-clamp recordings revealed the density of I_{CaL} did not differ between sexes for either age group for AT1R and wild-type (WT) mice. However, I_{CaL} density (in pA/pF) was significantly reduced in ventricular myocytes from 50-day male and female AT1R mice (at 0 mV, M: -4.2 ± 0.3, n=17 and F: -3.2 ± 0.3, n=6) compared to age-matched WT (M: -7.4 ± 0.4, n=20 and F: -6.8 ± 0.9, n=5) (all $p<0.05$). Similarly, I_{CaL} was significantly reduced in 6-month male and female AT1R myocytes (at 0 mV, M: -3.6 ± 0.2, n=15 and F: -3.0 ± 0.4, n=5) in comparison to WT cells (M: -5.9 ± 0.2 n=17 and F: 6.4 ± 0.2 n=10) (all $p<0.05$). Using real-time RT-PCR, we showed that ventricular $Ca_V1.2$ (L-type Ca^{2+} channel α-subunit) mRNA expression was decreased in 50-day and 6-month male and female AT1R mice compared to age- and sex-matched WT mice. Overall, the data indicates that the reduction in I_{CaL} and $Ca_V1.2$ in AT1R mice occurs independently of sex and cardiac remodelling. These alterations could contribute to the decreased cardiac contractility observed in AT1R mice.

2728-Pos

Sphingosine-1-Phosphate Regulates Volume-Sensitive Chloride Current in Ventricular Myocytes by Means of ROS

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We previously demonstrated that bacterial sphingomyelinase (SMase) activates a volume-sensitive current, $I_{Cl,swell}$, by a pathway that involves mitochondrial ROS production. SMase activity generates endogenous ceramides from sphingomyelin in the outer plasma membrane leaflet and, in turn, ceramides are metabolized to several sphingolipids, including sphingosine-1-phosphate (S1P). We tested whether ceramide metabolites are responsible for eliciting $I_{Cl,swell}$. Under isosmotic conditions that isolate anion currents, SMase-induced $I_{Cl,swell}$ was abrogated by blockade of ceramidase (converts ceramide to sphingosine) with D-*erythro*-MAPP (10 μM). SMase-induced $I_{Cl,swell}$ also was suppressed by inhibition of sphingosine kinase with DL-*threo*-dihydrosphingosine (10 μM). These data suggested that the ceramide metabolite S1P is likely to stimulate $I_{Cl,swell}$. As expected, exogenous S1P (500 nM) elicited an outwardly rectifying Cl^- current that was fully inhibited by the $I_{Cl,swell}$-specific blocker DCPIB (10 μM). As seen with SMase-induced $I_{Cl,swell}$, S1P-induced $I_{Cl,swell}$ was fully inhibited by the mitochondrial Complex I blocker rotenone (10 μM), which suppresses extramitochondrial ROS release by Complex III. In contrast to results with SMase, S1P-induced current was partially inhibited by blockade of NADPH oxidase (NOX) with apocynin (500 μM). These data indicate that S1P is a necessary component of SMase-induced $I_{Cl,swell}$ activation and that the action of exogenous S1P involves ROS from both mitochondria and NOX. Importantly, exogenous C_2-ceramide (2 μM), a synthetic short-chain ceramide, also elicits $I_{Cl,swell}$ even though C_2-ceramide is not metabolized to S1P in native cells. Thus, it seems likely that ceramides can elicit $I_{Cl,swell}$ via S1P and also by a distinct pathway and that both pathways converge at mitochondrial ROS.

2729-Pos

Modeling the Dynamic Currents Recorded under Action Potential-Clamp in Cardiac Myocytes

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The rhythm and shape of the cardiac action potential (AP) adapt on a moment-to-moment basis to our physical activity, emotional state, even our breathing. Underlying this exquisite adaptability is a constellation of ion channels and transporters that respond to extracellular and intracellular signals and the membrane voltage itself. At any moment, the dynamical behavior of the AP is governed by the sum of all ionic currents. Subtle changes in the kinetics or magnitudes of some currents can upset the precise choreography and generate, for example, early after-depolarization (EAD), which are often precursors to ectopic arrhythmias. Current cardiac AP models can reproduce the steady state AP properties but is less successful in accurately describing the transient/dynamic behavior of the AP such as those during adaptation and restitution, which are indicators of arrhythmias.

We hypothesized that this inaccuracy could be due to the experimental methods used to obtain the data for creating models. Current models are largely based on the experimental data obtained from traditional voltage clamp experiments using square pulse protocol and non physiological milieu. To overcome this limitation, we used the *self AP-clamp* technique to record the dynamic ionic currents under the cell's own AP in physiological milieu, which provides an accurate measure of the ionic currents experienced by the cell *in situ*. Here we report the modeling results describing the dynamic behavior of the ionic currents measured during the AP in guinea pig ventricular myocytes.

2730-Pos

The Role of Kinesin I and a Small Gtpase in the Forward Trafficking of Kv1.5 Channels

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Kv channels play important roles in the repolarization phase of the action potential in cardiac cells. The regulation of functional Kv1.5 surface expression has been reported to be modulated by retrograde trafficking through dynein motor but little is known about regulation by forward trafficking. Here, we use electrophysiological and immunocytochemical methods to investigate the mechanisms and regulation of anterograde trafficking of newly synthesized Kv1.5 channel proteins in cultured cells and in adult cardiomyocytes. Over-expression of a kinesin I isoform (Kif5b) increased outward K+ current by two fold in cultured cells stably expressing Kv1.5. This enhancement of Kv1.5 current by Kif5b was blocked by a six hour treatment with Brefeldin A. Over-expression of Kif5b increased Kv1.5 current additively with inhibition of endocytosis by p50 over-expression and dynamin inhibitory peptide. Deletion of a specific SH3-binding domain in Kv1.5 that is essential for internalization of the channel similarly enhanced Kif5b-induced Kv1.5 current. Expression of a dominant negative Kif5b mutant prior to induction of Kv1.5 in a tetracycline-inducible system almost completely blocked Kv1.5 current. These results

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