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