

TNF- $\alpha$ 's principal down-stream effectors and a major player in cardiac hypertrophy. Inhibition of NF- $\kappa$ B signaling, via an adenoviral expression of an IkappaB- $\alpha$  dominant negative mutant, prevented Ito,f reductions caused by S1P, TNF- $\alpha$  and PE. Taken together, the data suggests a linear pathway whereby PE promotes the shedding of TNF- $\alpha$ , which activates sphingosine kinase and elevates S1P, leading to activation of NF- $\kappa$ B and down-regulation of Ito,f. We will test this model in future experiments by assessing the effects of cardiac disease on Ito,f in knockout mice lacking sphingosine kinase and TNF- $\alpha$ .

#### 2746-Pos

##### Using Fluorescence Optical Mapping to Investigate the Electrophysiological Effects of Thienopyridines at the Tissue Level on Guinea Pig Papillary Muscle during Shock Induced Potential Changes

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Thienopyridines are frequently used drugs in the management of ischemic heart diseases or thrombotic events due to its antiplatelet as well as thrombolytic properties. Recently it was shown, that these compounds exert a negative inotropic effect in the isolated guinea pig heart. Using voltage clamp technique we could demonstrate a L-type calcium current impairment possibly underlying these effects. The aim of the study is to further elucidate the effects of thienopyridines on tissue level using high resolution optical mapping in guinea pig papillary. Our experimental setup features a 16 by 16 photodiode array with individual 256 custom-built current-to-voltage amplifiers. A frequency doubled continuous wave Neodymium-Yag laser (532 nm, 2 W) is used as excitation source. The tissue is stained by incubation with the voltage sensitive dye di-4-ANEPPS. The use of different objective-magnifications (10x, 20x, 40x, 63x) allows a multi-scale based analysis with resolutions up to 15 micrometers. The setup provides a detailed view on excitation propagation. In addition, the application of external electric field pulses during the depolarization phase of the tissue sample reveals local inhomogeneities in the membrane-potential distribution at the tissue surface (and possibly 2-3 cell layers below the surface).[1] This allows us to quantify the electrical inhomogeneity of the preparation. Experiments demonstrate the importance of optical membrane potential measurements, which provide new information unattainable with other methods.

[1] Windisch, H. et al. (2007): Quantification of shock-induced microscopic virtual electrodes assessed by subcellular resolution optical potential mapping in guinea pig papillary muscle. *J Cardiovasc Electrophysiol* 18(10), 1086-1094

#### 2747-Pos

##### Do Mouse Epicardial Action Potentials Present Phase 2?

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Mouse ventricular action potentials (AP) recorded from acute dissociated cardiac myocytes lacks of phase 2. In order to evaluate if this is always true for all the muscular layers within the ventricular wall, we decided to perform optical and electrophysiological experiments to evaluate the time course of APs in the subepicardial layer of intact perfuse mouse hearts. Epicardial APs show a typical spike and dome morphology present in other non-rodent mammalian species. The APs recorded optically by means of Pulsed Local Field Fluorescence Microscopy and the potentiometric dye Di-8-ANEPPS show a very similar time course when compared to the one obtained with microelectrodes. However, the phase 2 of the optically recorded APs is more depolarized in comparison with the electrically recorded one suggesting a larger contribution of the t-system to the epicardial APs. In order to evaluate the molecular mechanisms involved in the genesis of phase 2 APs in mouse epicardium we performed experiments to evaluate the role of intracellular  $\text{Ca}^{2+}$  release on the time course of APs. Interestingly, both ryanodine/thapsigargin treatment and perfusion with caffeine significantly decreased the contribution of phase 2 to the APs. Finally we evaluate the hypothesis that intracellular  $\text{Ca}^{2+}$  is translated in AP changes by an activation of influx of  $\text{Na}^{+}$  through the Na/Ca exchanger in the forward mode. Experiments where extracellular  $\text{Na}^{+}$  was replaced by increasing concentration of  $\text{Li}^{+}$  induce a significant decrease of the AP phase 2. Moreover, two known blockers, KB-R7943 (KBR) and SEA0400 (SEA) also dramatically decrease the contribution of phase 2 to the AP time course. Our results indicate that mouse epicardial AP displays a significant phase 2 that is generated by an influx of  $\text{Na}^{+}$  through the Na/Ca exchanger. Supported by NIH R01-HL-084487 to AE.

#### 2748-Pos

##### Actions of ATP on Guinea-Pig SA Node

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ATP is well established as a co-transmitter in sympathetic nerves supplying smooth muscle. In the heart, ATP has been shown to increase pacemaker

activity in amphibian preparations but little is known about its effects on pacemaker activity in mammals. The aim of these experiments was to investigate the actions of ATP and its analogues on guinea-pig SA node. Alpha,beta methylene ATP (abMeATP) caused a concentration dependent increase in the rate of beating of isolated atrial preparations over the range 0.3 to 30 micro M. ATP also caused a concentration-dependent increase in the rate of beating in isolated atrial preparations over the range 3 to 100 micro M, provided that adenosine receptors were antagonised by 1,3-dipropyl-8-cyclopentylxanthine. In myocytes isolated from guinea-pig SA node, abMeATP (1 and 10 micro M) caused an increase in the rate of firing of spontaneous action potentials. In the same range of concentrations, abMeATP increased the I(f) current activated by hyperpolarization in voltage-clamped SA node myocytes. This effect of abMeATP was prevented when SA node myocytes were loaded with the calcium chelator BAPTA (by exposure to the AM ester). These observations are consistent with an action of abMeATP on pacemaker activity in guinea-pig SA node mediated by calcium entry via P2X receptors. This calcium entry is expected to activate calcium-stimulated adenylyl cyclase (which we have shown to be present in these cells) leading to increased levels of cAMP and enhanced activation of I(f). Calcium entry via P2X receptors may also have additional effects on other pathways involved in pacemaker activity. Actions on P2X receptors are expected to contribute to the observed effects of ATP in SA node when the inhibitory effects of adenosine (a possible breakdown product of ATP) are antagonised.

## Channel Regulation & Modulation II

#### 2749-Pos

##### Mutations in Extracellular Domains Reverse $\text{Zn}^{2+}$ Activation of Human Epithelial $\text{Na}^{+}$ Channels

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Epithelial  $\text{Na}^{+}$  channels (ENaCs) mediate apical  $\text{Na}^{+}$  entry into epithelial cells in kidney, lung and distal colon, playing a critical role in regulation of body fluid volume homeostasis. Several divalent metals modulate ENaC activity in a species-dependent manner. We examined the effect of extracellular  $\text{Zn}^{2+}$  on human  $\alpha\beta\gamma$ ENaCs expressed in *Xenopus* oocytes and investigated the underlying mechanisms. External  $\text{Zn}^{2+}$  increased the whole-cell currents of human ENaCs with a bell-shaped dose response similar to the reported response of mouse ENaC. A peak activation was observed at 100  $\mu\text{M}$  with lower or higher  $[\text{Zn}^{2+}]$  being less effective. As previously reported for mouse ENaCs,  $\text{Na}^{+}$  self-inhibition response of human ENaCs was nearly eliminated in the presence of 100  $\mu\text{M}$   $\text{Zn}^{2+}$ , supporting the notion of relieving  $\text{Na}^{+}$  self-inhibition as the major mechanism for  $\text{Zn}^{2+}$  stimulation of human ENaC currents. We found that mutations of His<sup>233</sup> in  $\gamma$  subunit, a residue required for  $\text{Na}^{+}$  self-inhibition, converted human ENaC into a  $\text{Zn}^{2+}$ -inhibited channel with an estimated inhibitory constant of 1 mM. This observation contrasts a previous report of a complete loss of response to  $\text{Zn}^{2+}$  in mouse ENaCs bearing homologous mutations. Mutations at two extracellular Cys residues in  $\alpha$ ENaC but not their homologous residues in  $\gamma$ ENaC also reversed the stimulatory effect of  $\text{Zn}^{2+}$  on human ENaCs. This phenol type cannot be attributed to an absence of  $\text{Na}^{+}$  self-inhibition, because the Cys mutants in fact showed enhanced responses to  $\text{Na}^{+}$ . Our results suggest that activation of human ENaCs by extracellular  $\text{Zn}^{2+}$  requires different structural elements from those in mouse ENaCs despite the similar dose response.

#### 2750-Pos

##### Polycystin-2 Contains an Unpaired EF-hand Motif which May Serve as a $\text{Ca}^{2+}$ -Sensitive Regulator of Polycystin-2 Channel Activity

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Autosomal dominant polycystic kidney disease (ADPKD) is the most common, monogenic cause of kidney failure in humans. Most cases of ADPKD are linked with mutations in polycystin-1 (PC1) and polycystin-2 (PC2). PC2 is a calcium ( $\text{Ca}^{2+}$ ) permeable channel in the TRP channel family. Deletion of the C-terminus of PC2 alters  $\text{Ca}^{2+}$ -signaling; the most common pathogenic mutations in PC2 are premature truncations. We previously showed that this tail consists of three functional regions: an unpaired EF-hand domain (PC2-EF), an oligomeric coiled coil domain, and a linker connecting them. We hypothesize that the EF-hand serves as a  $\text{Ca}^{2+}$ -sensor/switch, and show that PC2 undergoes  $\text{Ca}^{2+}$ -induced conformational changes by NMR, CD, and SAXS. We have solved the NMR structure of  $\text{Ca}^{2+}$ -bound PC2-EF and have identified residues with chemical shift changes upon  $\text{Ca}^{2+}$ -titration. PC2-EF contains a novel unpaired EF-hand fold which may have evolved from a canonical paired EF-hand found in invertebrate PC2 homologs. Human PC2-EF contains a divergent helix-loop-helix in place of a second EF-hand.