TNF-alpha's principal down-stream effectors and a major player in cardiac hypertrophy. Inhibition of NF-kappaB 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-kappaB 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-Anneps 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 Ca2+ 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 Ca2+ is traduced in AP changes by an activation of influx of Na+ through the Na Ca exchanger in the forward mode. Experiments where extracellular Na+ was replaced by increasing concentration of 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 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. Apha,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 Zn^{2+} Activation of Human Epithelial Na^+ Channels

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Epithelial Na^+ channels (ENaCs) mediate apical 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 Zn^{2+} on human $\alpha\beta\gamma$ENaCs expressed in *Xenopus* oocytes and investigated the underling mechanisms. External 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 μM with lower or higher [Zn^{2+}] being less effective. As previously reported for mouse ENaCs, Na^+ self-inhibition response of human ENaCs was nearly eliminated in the presence of 100 μM Zn^{2+}, supporting the notion of relieving Na^+ self-inhibition as the major mechanism for Zn^{2+} stimulation of human ENaC currents. We found that mutations of His^{233} in γ subunit, a residue required for Na^+ self-inhibition, converted human ENaC into a 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 Zn^{2+} in mouse ENaCs bearing homologous mutations. Mutations at two extracellular Cys residues in αENaC but not their homologous residues in γENaC also reversed the stimulatory effect of Zn^{2+} on human ENaCs. This phenol type cannot be attributed to an absence of Na^+ self-inhibition, because the Cys mutants in fact showed enhanced responses to Na^+. Our results suggest that activation of human ENaCs by extracellular 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 Ca2+-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 (Ca2+) permeable channel in the TRP channel family. Deletion of the C-terminus of PC2 alters Ca2+-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 Ca2+-sensor/switch, and show that PC2 undergoes Ca2+-induced conformational changes by NMR, CD, and SAXS. We have solved the NMR structure of Ca2+-bound PC2-EF and have identified residues with chemical shift changes upon Ca2+-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.

Temperature dependent steady state NOE experiments and NMR linewidth measurements indicate increased molecular motion in the EF-hand consistent with a proposed role for PC2-EF as a Ca2+-sensitive regulator. Structure-based sequence conservation analysis reveals a conserved hydrophobic pocket in this region, where PC2-EF may mediate Ca2+-dependent protein interactions. Using results of our structural studies we have examined the role of the EF-hand and coiled coil on PC2 channel function in single-channel lipid bilayers. Our results suggest that the coiled coil regulates PC2 by serving as an homo-oligomerization motif, whereas the EF-hand modulates the Ca2+-dependence of PC2 channel activity. Based on our results we propose a mechanism of regulation of the Ca2+-dependence of PC2 channel activity by PC2-EF.

2751-Pos

New Channels in the Outer Mitochondrial Membrane

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Mitochondria are the "power stations" of eukaryotic cells. Beside this they play essential roles for the metabolism and physiology of cells and are a central point of apoptosis regulation. Mitochondria are also involved in calcium homeostasis. Due to endosymbiontic engulfment mitochondria are surrounded by two membranes. While the regulation of the metabolite flux across the inner membrane (IMM) is extensively characterised, it has been generally assumed that the outer membrane (OMM) functions only as a barrier for molecules larger than 3 kDa. But recent studies demonstrate that the metabolite flux between the cytosol and the different compartments of mitochondria is regulated at the level of the outer membrane.

Three pore forming proteins are up to now known in the outer membrane. Two of them are essential and involved in protein transport and insertion into OMM. These are Tom40 and Sam50/Tob55. The third one is the non-essential metabolite pore VDAC (voltage-dependent anion channel). The none lethal phenotype of VDAC knockouts discloses that it is the sole metabolite conducting pore in the OMM and the presence of other non-identified channels in the OMM is very likely.

The OMM proteome contains more than 112 proteins and only for less than 10 % of them the function is known. By electrophysiological screening of highly pure $OMM_{vdac\ \Delta}$ vesicles it was possible to identify at least four distinct membrane pores. In a first bioinformatical attempt using specific parameters like the isoelectric point or second structure prediction programs we identified eight potential channel candidate proteins.

2752-Pos

Evidence for Lateral Budding and Voltage Dependence of a Proteo-Lipid Channel

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The pro-apoptotic protein, Bax, and the sphingolipid, ceramide, can individually form channels in phospholipid membranes. When combined, they permeabilize membranes in a synergistic way, indicating the formation of a combined channel structure. Nanomolar quantities of $LaCl_3$ disassemble ceramide channels completely but 10 micromolar $LaCl_3$ is needed to convert one large Bax-ceramide channel into a population of virtually identical channels. These channels exhibit voltage-dependent closure or disassembly. Some of the channels can be reassembled by reducing the voltage or applying an opposite potential but cycles of voltage-dependent closure and reopening quickly result in loss of conductance. There are indications that the transformation of the one large channel into a population of small channels occurs by lateral budding in the plane of the membrane. Over 100 such small channels were formed in one experiment and the application of an elevated potential resulted in a long staircase of virtually identical conductance decrements. These results open a window into phenomenology that, to our knowledge, has not been described previously.
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2753-Pos

A Kinetic Model of Ion Channel Electrophysiology: Incorporating Bilayer-Mediated Effects of Agonists and Anesthetics on Protein Conformational Transitions

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The time- and concentration dependence of agonist-induced ion currents through postsynaptic receptors is often remarkably complex, involving desensitization and deactivation on multiple time scales, as is the modulation of these currents by other solutes such as anesthetics. Traditional kinetic models have involved agonist binding and conformational transitions among a very large manifold of protein conformational states engineered to reproduce the complexity of a particular set of electrophysiological results. However, independent experimental evidence for the hypothetical additional conformational states (beyond the minimal set of resting, conducting and desensitized) is essentially nonexistent, nor is there any model-independent way of estimating the values of the associated kinetic parameters. We propose an alternative model that includes only these three essential states while additionally incorporating the adsorption of agonist and nonbinding compounds such as anesthetics to the bilayer in which these intrinsic membrane proteins are embedded [R. S. Cantor et al., *Soft Matter*, 2009, **5**, 3266]. Solute adsorption alters bilayer physical properties, which in turn distorts the protein conformational free energy landscape, and thus alters the rate constants of protein conformational transitions. The complexity of the predicted ion currents - often well approximated as sums of exponentials - then arises from the time-dependence of solute adsorption, resulting in strongly time-dependent transition rate ''constants''. If only nonbinding solutes are present, the model simplifies considerably. For this special case, best fits of predicted current traces with respect to a small set of parameters are in excellent agreement with fast-perfusion electrophysiological studies of recombinant $GABA_A$ receptors [R. Haseneder et al., *Eur. J. Pharm.*, 2002, **451**, 43] in which currents are induced in the absence of agonist by a broad range of supraclinical concentrations of isoflurane and sevoflurane.

2754-Pos

KChiP2 Stabilizes Kv4 Protein Expression and Cell Surface Retention to Control Cardiac Ito Channel Densities

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The myocardial transient outward current (I_{to}) is encoded by voltage-gated potassium (Kv) channel α-subunits of the Kv4 subfamily, together with the cytosolic accessory subunit, KChIP2. Targeted deletion of KChIP2 (KChIP2-/-) or Kv4.2 (Kv4.2-/-) eliminates I_{to} in adult mouse ventricular myocytes. Heterologous co-expression with KChIP2 increases Kv4.2 current densities and results in a relative shift in Kv4.2 from a perinuclear localization to the cell surface, leading to the suggestion that KChIP2 alleviates retention of assembled Kv4 channels in the endoplasmic reticulum (ER) and promotes forward trafficking. To explore these hypotheses, a putative RXR-type ER-retention motif at residues 35-RKR-37 in Kv4.2 was mutated (Kv4.2AAA), and the functional consequences of this construct on Kv4.2 expression in human embryonic kidney-293 (HEK) cells were explored. Mean ± SEM peak Kv4.2 current densities in cells expressing Kv4.2AAA (316 ± 50 pA/pF) were significantly ($p=0.025$) higher than in cells expressing wild type Kv4.2 (174 ± 20 pA/pF). Surprisingly, however, adenoviral expression of Kv4.2AAA in Kv4.2-/- myocytes resulted in peak Kv current densities (86 ± 9 pA/pF) that were not significantly different from the peak Kv currents (72 ± 9 pA/pF) in Kv4.2-/- cells infected with wild type Kv4.2. Heterologous expression of a charge-conservative mutant, Kv4.2KKK, in which arginines 35 and 37 were mutated to lysines (Kv4.2KKK), resulted in Kv4.2 currents (172 ± 25 pA/pF) that were indistinguishable from wild type currents, demonstrating that the presence of charged residues in the Kv4.2 N-terminus affects channel gating, not channel trafficking. Biochemical studies revealed no differences in the surface expression of the Kv4.2AAA mutant and wild type Kv4.2, and the surface expression of both constructs was increased dramatically upon co-expression of KChIP2. The results of further biochemical studies suggest that KChIP2 functions to increase the retention of Kv4.2 channels at the cell surface.

2755-Pos

The Na^+-Activated Potassium Channel Slack Shares a Similar Na^+ Coordination Site with Kir3 Channels

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Characteristics of Na^+ activated potassium channel (Slack or Slo2.2) currents, including high conductance, rundown, regulation by Na^+, Cl^- and phosphorylation have long been reported but underlying mechanisms remain unknown. Here we report identification of a sodium regulatory site in the RCK2 domain of Slack channels by screening the C-terminus with the conserved sodium coordination motif of Kir channels. While the charge preserving D818E mutation exhibited similar Na^+ sensitivity as the wild-type Slack channel, both