elevations of fNADH, both in time and space. During regional ischemia, occasional breakthroughs occurred, most of them along the boundary between ischemic and normoxic tissue. During low-flow reperfusion, the number of breakthroughs within the ischemic zone increased dramatically as well as the incidence of ventricular fibrillation (compared to ischemia and normal flow conditions). CONCLUSIONS: The inter-dependence of local activation patterns and local myocardial metabolism makes parallel imaging of fNADH and TMP an essential tool for understanding the mechanisms of arrhythmias caused by ischemia and reperfusion.

2741-Pos

The Transient Outward Current Ito Promotes Early After depolarizations

Yuanfang Xie[1], Zhenghang Zhao[2], James N. Weiss[1], Zhilin Qu[1], Lai-Hua Xie[2].
[1]UCLA, Los Angeles, CA, USA, [2]New Jersey Medical School, Newark, NJ, USA.

The transient outward current (Ito) plays important roles in action potential (AP) morphology and arrhythmogenesis in cardiac diseases, such as ischemia and the Brugada syndrome. It is well accepted that early afterdepolarizations (EADs) occur under conditions of reduced repolarization reserve, which can result from either increased inward currents or reduced outward currents. Here we show the novel finding that Ito, an outward current, promotes EADs in rabbit ventricular myocytes, raising the question: how does an outward current promote EADs? To answer this question, we carried out experimental studies in isolated rabbit ventricular myocytes, theoretical analysis, and computer simulations. In myocyte experiments, exposure to 0.2-1 mM H2O2 at slow pacing rates induced EADs, which were eliminated by selectively blocking Ito with 2 mM 4-aminopyridine. Pre-treating myocytes with 4-aminopyridine prolonged AP, but likewise prevented H2O2-induced EADs. Voltage-clamp experiments showed that besides promoting late ICa,L and late INa, H2O2 also increased the maximum conductance, slowed the inactivation and accelerated the recovery from inactivation of Ito. When the cells were clamped with AP morphologies corresponding to the absence and presence of Ito, Ito significantly enhanced the Ca current, promoting its reactivation as the mechanism induced EADs. In a computer model of the rabbit ventricular AP, we also showed that the presence of Ito promoted EADs. The rate of Ito inactivation played a critical role: if too fast, no EADs occurred, and if too slow, AP duration became too short and no EADs occurred either. The underlying dynamical mechanisms were revealed by bifurcation theory of EADs previously developed by our group (Tran et al, Phys. Rev. Lett. 2009; 102:258103).

2742-Pos

Potassium Channels in Fetal Human Cardiomyocytes Compared to Rat and Rabbit

Johan Brask[1], Christian Danielsson[2], Christer Sylvén[3], Fedrik Elinder[1].
[1]Linkopings Universitet, Linkoping, Sweden, [2]Karolinska Institutet, Stockholm, Sweden, [3]Karolinska University Hospital, Stockholm, Sweden.

Some side effects of medical drugs are caused by block of cardiac ion channels leading to cardiac arrhythmia. Because of different sets of ion channels in rat and rabbit, and adult and fetal humans, the conclusions on side effects are difficult to translate from species to species and even within one species. This study investigates the differences in potassium currents during the most vulnerable period of the development of the heart and compares the human fetal cardiomyocytes with rat and rabbit to understand the differences in drug effects on the heart's function between the species.

In rat we have used two time points for the study. Potassium channels at the embryonic day 11 (E11), the most vulnerable time point for the heart, are compared with E15, a much less vulnerable time point. E11 is also compared with E10 from rabbit and fetal human cardiomyocytes. The fetal human cardiomyocytes are from week 5 to 9, also in the risk period. We have studied the potassium currents IKr, IKs, and IK1 by the patch-clamp technique. We have also investigated the importance of the currents in generating action potentials.

2743-Pos

Action Potential Duration Modulation by Activation Sequence in Rat Vs. Pig Myocardium

Richard D. Walton, Ed White, Olivier Bernus.
University of Leeds, Leeds, United Kingdom.

Dispersion of the cardiac action potential duration (APD) is known to influence the susceptibility of cardiac tissue to arrhythmias. Several experimental studies have revealed that APD can be modulated by the activation sequence. Our lab has recently shown strong correlations between APD and activation time (AT) in hearts from small rodents. However, a recent computational study indicated that the magnitude of such APD modulation may not be consistent across species. Therefore, the present study sought to compare experimentally APD modulation by activation sequence in rat and pig.

Optical imaging using the voltage-sensitive dye Di-4-ANEPPS was performed in Langendorff perfused rat hearts (n=4) and coronary perfused pig left ventricular slabs (n=5). The left ventricular mid-free wall was paced at 6Hz (rat) and 2Hz (pig), close to their intrinsic heart rate, and optical action potentials were acquired for 5s.

The mean APD near the pacing site (at 4ms AT) was 44.9 $\pm$ 9.4 ms in rat and 142.2 $\pm$ 11.3 ms in pig. A significant decrease of APD was revealed at larger AT in rat (37.2 $\pm$ 9.5 ms at 10ms AT, P0.05). Plotting APD as a function of AT revealed a linear correlation of APD with AT. Slope analysis revealed a decreasing trend in rats (mean slope $= -0.79 \pm 0.26$) whilst pigs showed no such modulation of APD (mean slope $= 0.29 \pm 0.22$) Heterogeneity, defined as the APD covariance over the whole field of view, was 0.10 in rat and 0.05 in pig ($P<0.05$).

In conclusion, APD can be strongly modulated by the activation sequence in hearts from small rodents whilst this modulation is absent in pig myocardium. This study emphasises the importance of APD heterogeneity induced by activation sequence and differences between species.

2744-Pos

Pressure Puff Induced Calcium Signals in Voltage Clamped Cardiomyocytes

Brooke Damon[1], Lars Cleemann[2], Martin Morad[2].
[1]Medical University of South Carolina, Charleston, SC, USA, [2]University of South Carolina, Columbia, SC, USA.

I_{Ca}-gated release of Ca^{2+} from the SR is the dominant mechanism mediating cardiac E-C coupling. On the other hand, in the absence of Ca^{2+} entry, supplemental Ca^{2+} release may be activated mechanically, either from the SR secondary to nitrosylation of RyRs or from mitochondria as a direct effect of puff-induced shear force. Here we have probed the puff-induced Ca^{2+} release in voltage-clamped cardiomyocytes, where the Ca^{2+}-indicator rhod-2 was targeted to mitochondria by: a) staining with the AM-form of the dye for 45 minutes, b) incubating without dye for 24-30 hours and c) dialyzing with dye-free internal solution through the voltage-clamp pipette for 20 minutes before initiating measurements. In such cells, which remained responsive for periods as long as 2 hours, we measured relatively slow (~1s) puff-induced deceases in fluorescence suggestive of mitochondrial Ca^{2+} release as previously found in non-dialyzed and permeabilized cells (Belmonte and Morad, 2008, J Physiol 586:1376). To clarify the $[Ca^{2+}]_i$- signaling under these conditions, our experimental paradigm included activation of I_{Ca} both at the beginning and end of a 2 second priming interval where the cell was exposed to control solution, 10 mM Caffeine or zero Na^+ (and high K^+). The caffeine-induced Ca^{2+} signal was biphasic with internal solution containing 0.2 or 14mM EGTA, generating I_{NaCa} only in 0.2 but not in 14mM EGTA during the rapid initial rise of Ca^{2+}, suggesting that the maintained component of the Ca^{2+} signal arises from a confined and most likely mitochondrial space, not detected by NCX. We conclude that patch clamped Rhod-2 loaded myocytes that were washed overnight and dialyzed for 1-2 hour with 14mM EGTA produced reliable mitochondrial Ca^{2+} signals supporting the finding that the PF-triggered Ca^{2+}-transients are caused by mitochondrial Ca^{2+} release.

2745-Pos

Regulation of the Transient Outward Potassium Current I_{tof} in Cardiac Hypertrophy by Sphingosine-1-Phosphate Signaling

Brian K. Panama[1], Desiree Latour[1], Lorrie Kirshenbaum[2], Steffen-Sebastian Bolz[1], Peter H. Backx[1].
[1]University of Toronto, Toronto, ON, Canada, [2]University of Manitoba, Winnipeg, MB, Canada.

The fast transient outward potassium current (Ito,f), which is carried by voltage-gated Kv4.2 and Kv4.3 potassium channels and auxiliary subunit KChIP2, plays a critical role in early repolarization of the cardiac action potential. Ito,f and its gene products are strongly down-regulated in cardiac hypertrophy and disease, leading to altered excitation-contraction coupling and electrical activity as well as hypertrophy. Despite the importance of Ito,f in normal and diseased hearts, the regulation of Ito,f remains poorly understood. Studies have shown that the biologically active sphingolipid, sphingosine-1-phosphate (S1P), induces cardiac hypertrophy. In addition, the inflammatory pro-hypertrophic cytokine TNF-alpha, which decreases Ito,f, activates sphingosine kinase 1, the highly regulated enzyme that produces S1P. Therefore, we investigated the role of TNF-alpha and S1P signaling in mediating the down-regulation of Ito,f. In cultured neonatal myocytes, the TNF-alpha inhibitor etanercept attenuated reductions in Ito,f current density that were caused by the hypertrophic agonist phenylephrine (PE). Inhibition of sphingosine kinases by dimethylsphingosine prevented reductions in Ito,f that were caused by PE. Furthermore, application of S1P reduced Ito,f current density and caused hypertrophy. To interrogate the down-stream events involved in TNF-alpha/S1P- induced reductions in Ito,f, we focused on NF-kappaB since it is one of

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

Dieter Platzer, Brigitte Pelzmann, Klaus Zorn-Pauly.
Medical University Graz, Graz, Austria.

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?

Marcela Ferreiro, Azade Petrosky, Dmitro Korneyeyev, Ariel L. Escobar.
UC Merced, Merced, CA, USA.

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

Derek A. Terrar, Paul Mattick, Stevan Rakovic.
University of Oxford, Oxford, United Kingdom.

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

Jingxin Chen, Katie L. Winarski, **Shaohu Sheng**.
University of Pittsburgh, Pittsburgh, PA, USA.

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

Andjelka Celic, Edward T. Petri, Michael E. Hodsdon, Titus J. Boggon, Barbara E. Ehrlich.
Yale University, New Haven, CT, USA.

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.