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

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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

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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

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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

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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

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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