1319-Pos Board B163

Ceramide Activates $I_{Cl,swell}$ in Rabbit Ventricular Myocytes via Mitochondrial ROS Production

Frank J. Raucci Jr., Clive M. Baumgarten.
Medical College of Virginia, Virginia Commonwealth University, Richmond, VA, USA.

We previously showed that $I_{Cl,swell}$ is activated by ROS generation via NADPH oxidase (NOX) and the mitochondrial electron transport chain (ETC). Sphingolipid signaling is implicated in channel regulation. Here we examined the role of ceramide in the modulation of $I_{Cl,swell}$. Under isosmotic conditions, the addition of exogenous C_2-ceramide (C_2-Cer, 2 ∝M) increased Cl^- current density by 0.7 ± 0.1 pA/pF at +60 mV after 10 min ($n = 11$, $P<0.01$). DCPIB (10 ∝M), a highly selective $I_{Cl,swell}$ antagonist, inhibited C_2-Cer-induced $I_{Cl,swell}$ by 76 ± 8% ($n = 6$, $P<0.01$). The inactive analogue C_2-H_2Cer (2 ∝M) failed to stimulate $I_{Cl,swell}$ ($n = 6$). Bacterial sphingomyelinase (SMase, 0.03 U/mL) was used to elicit endogenous ceramide production. SMase increased $I_{Cl,swell}$ by 1.1 ± 0.1 pA/pF after 14 min ($n = 30$, $P<0.01$). This activation was inhibited by DCPIB (78 ± 6%, 10 ∝M, $n = 7$ and 81 ± 6%, 30 ∝M, $n = 4$) and tamoxifen (116 ± 16%, 10 ∝M, $n = 5$). Next we identified the source of ROS. Exposure to the NOX-specific inhibitor apocynin (500 ∝M, 10 min) failed to suppress SMase-induced $I_{Cl,swell}$ ($n = 9$), whereas we previously showed apocynin blocks activation of $I_{Cl,\ swell}$ on swelling and stretch. Diphenyleneiodonium (60 ∝M), a flavoprotein oxidase antagonist that suppresses both NOX and ETC Complex I, fully inhibited SMase-induced $I_{Cl,swell}$ after 20 min (100 ± 14%, $n = 4$, $P<0.01$). Rotenone (10 ∝M), a specific ETC Complex I inhibitor, also abrogated the SMase-induced activation of $I_{Cl,swell}$ after 20 min (110 ± 18%, $n = 5$, $P<0.05$). These data indicate that there is a ceramide-sensitive component of $I_{Cl,swell}$, and it is regulated by mitochondrial ROS. Ceramide signaling may modulate $I_{Cl,swell}$ in cardiac disease.

1320-Pos Board B164

The persistently beating hagfish heart

Lars Cleemann[1,2], Sarah Haviland[1,2], Ronnie Dalsgaard[3,2], Martin Morad[1,2].
[1]Georgetown University, Washington, DC, USA, [2]Mount Desert Island Biological Laboratory, Salsbury Cove, ME, USA, [3]Danish Technicasl University, Copenhagen, Denmark.

To elucidate the evolution of autonomic cardiac reflexes in primitive chordates, we measured isometric force and trans-gap action potentials in ventricular and atrial strips (diameter 0.6-0.8 mm) from the systemic heart of the Atlantic hagfish, *Myxine glutinosa*. All parts of the heart paced spontaneously with a frequency, that at all temperatures was ~30% faster for atrial than for ventricular tissue. Active force development increased with stretch, and remained high as passive force rose concurrently. In spite of the low blood pressure of hagfish, the maximal contractile force (~60 mN/mm^2) was comparable to that of higher vertebrates. Electrical stimulation at frequencies higher than the inherent, demonstrated capture and refractoriness consistent with the atrial pacing observed in the intact heart. The isometric twitches that developed during the long lasting plateaus of the action potentials were relatively insensitive to $[Na^+]_o$, $[Ca^{2+}]_o$, epinephrine, and carbachol, but were promptly abolished by depolarization by $[K^+]_o$. Beat kinetics showed no indication of releasable intracellular Ca^{2+} stores. KCl-contractures developed extremely slowly and were insensitive to epinephrine. In addition, we report a partial sequencing (1077 bp) of the cardiac Na^+-Ca^{2+} exchanger using degenerate CODEHOP primers determined from known marine species Na^+-Ca^{2+} exchangers. It was surprising, therefore, that the sequencing of the Na^+-Ca^{2+} exchanger showed both a putative PKA site and indication of the typical ''cardiac'' splicing pattern of NCX1 (exon pattern ACDEF). The results are compared to similar measurements from tunicates, sharks, and higher vertebrates. The most surprising finding is the ability of all parts of the heart to generate pacemaker activity at a frequency close to that of the heart *in situ.*

1321-Pos Board B165

The Role Of Depolarizing And Repolarizing Currents In The Induction Of Early Afterdepolarizations During Acute Hypoxia In Ventricular Myocytes

William A. Macdonald[1], Namit Gaur[2], Yoram Rudy[2], Livia C. Hool[1].
[1]The University of Western Australia, Crawley, Australia, [2]Washington University, St Louis, MO, USA.

Coronary occlusion is associated with acute hypoxia and increased catecholamine levels. Acute hypoxia (decreasing PO_2 from 150 to 17mmHg) is not energy limiting but can alter the function of ion channels. Hypoxia decreases the transient Na^+-current ($I_{Na\text{-}T}$), the basal L-type Ca^{2+} channel current ($I_{Ca\text{-}L}$) and the slow component of the delayed rectifier K^+-current (I_{Ks}), without effecting the rapid component (I_{Kr}). Hypoxia also increases the persistent Na^+-current ($I_{Na\text{-}P}$) and the sensitivity of $I_{Ca\text{-}L}$ and I_{Ks} to the β-adrenergic receptor agonist isoproterenol (Iso). The net effects of acute hypoxia and catecholamines on the cardiac action potential are not known.

We incorporated all published data reporting the effects of acute hypoxia on cardiac ion channels into the Luo-Rudy model. Results from the model were compared with experimental data we obtained from myocytes.

Our modelled data predicts acute hypoxia, in the absence of Iso, has little effect on resting membrane potential (RMP), action potential peak (APP) or action potential duration (APD). Hypoxia alone did not trigger EADs. When we added 0.6nM Iso to $I_{Ca\text{-}L}$ during hypoxic conditions, APD was prolonged and EADs were generated. Repeating this for I_{Ks} did not alter APD or generate EADs. When we modelled the effects of hypoxia on $I_{Ca\text{-}L}$ and I_{Ks}, APD was prolonged and EADs were generated, suggesting any anti-arrhythmic effect of I_{Ks} is small and that $I_{Ca\text{-}L}$ effects predominate.

Experimental data confirmed that acute hypoxia alone did not markedly alter RMP, APP or APD. In the presence of 3nM Iso, hypoxia significantly increased APD by 20% and induced EADs and spontaneous tachycardia. We conclude that during acute hypoxia, EADs are induced predominantly as a result of increased sensitivity of the L-type Ca^{2+} channel to β-adrenergic receptor stimulation.

1322-Pos Board B166

Increasing Cardiac Contractility after Myocardial Infarction Exacerbates Cardiac Injury and Pump Dysfunction

Hongyu Zhang[1], Xiongwen Chen[1], Erhe Gao[2], Scott Macdonnell[1], Naser Jaleel[1], David M. Harris[2], Hiroyuki Nakayama[3], Xiaoying Zhang[1], Yingxin Li[1], Mingxin Tang[1], Remus Berretta[1], Walter J. Koch[2], Jeffery D. Molkentin[3], Steven R. Houser[1].
[1]Temple University, Philadelphia, PA, USA, [2]Thomas Jefferson University, Philadelphia, PA, USA, [3]Children's Hospital Medical Center, Cincinnati, OH, USA.

Myocardial infarction (MI) induces cardiac remodeling and leads to poor cardiac pump function. Increasing the contractility of the surviving myocytes is one therapy thought to improve the function of failing heart. However, the excess sympathetic activity in heart failure coupled with increased inotropic therapy could induce cell death and exacerbate cardiac dysfunction. In this study we tested the effects of increasing Ca^{2+} influx through the L-type Ca^{2+} channel (LTCC) in the post MI heart on myocyte contractility, cardiac function and remodeling.

Methods: Double transgenic mouse lines with inducible (Tet-off) and cardiac myocyte specific (αMHC promoter) expression of the β2a subunit of the L-type Ca^{2+} channel were used. MI was produced by permanent ligation of the left anterior descending coronary artery. In-vivo cardiac function was measured with the Visual Sonics Velvo 770 system. Myocytes were isolated and LTCC Current ($I_{Ca\text{-}L}$) and fractional shortening (FS) were measured in wild type (WT) and β2a hearts before, and 3 weeks after MI. **Results:** Echo measurements showed decreased heart function in both groups after MI, but β2a mice had significantly lower ejection fraction (EF) and larger left ventricular internal diameter (LVID) than WT mice (EF: 24.7% vs.42.6%; LVID: 5.2 mm vs. 4.2 mm). $I_{Ca\text{-}L}$ and FS were greater in uninfarcted myocytes from β2a vs WT mice ($I_{Ca\text{-}L}$ 24.5 ± 1.7 vs.13.7 ± 1.8 pA/pF FS: 12.31 ± 1.15% vs. 9.0 ± 0.5%). 3 weeks after MI, $I_{Ca\text{-}L}$ and FS in myocytes from both groups were decreased, but β2a myocytes still had significantly higher $I_{Ca\text{-}L}$ and FS than in WT myocytes (β2a vs. Control: 18.37 ± 1.90 vs.11.57 ± 0.77 pA/pF and 12.05 ± 0.74% vs. 7.16 ± 0.99%). **Conclusions:** Increasing Ca^{2+} influx through the LTCC in the post MI heart increases myocyte contractility but depresses cardiac pump function, possibly by increasing myocyte death.

1323-Pos Board B167

Chemical Ablation Of Purkinje Fibers Diminishes Spontaneous Activity In A Rat Model Of Regional Ischemia And Reperfusion

Matthew W. Kay, Luther M. Swift, Huda Asfour, Craig Forleiter, Marco A. Mercader, Narine A. Sarvazyan.
The George Washington University, Washington, DC, USA.

Spontaneous activity and arrhythmias associated with acute local ischemia and reperfusion were studied in isolated Langendorff-perfused hearts from healthy Sprague-Dawley rats (n=16). Epicardial fluorescence imaging of transmembrane potential and NADH were used to relate sources of electrical activity to changes in mitochondrial redox state caused by local ischemia. The left anterior descending coronary artery was cannulated and the flow of perfusate to the cannula was controlled by a high-pressure/low-flow HPLC pump. Studies were conducted using a local ischemia/reperfusion protocol that consisted of 10 min of normal flow, 20 min of regional LV ischemia, followed by 20 minutes of reduced flow reperfusion, and then 20 min of normal flow reperfusion. Control hearts (n=9) were compared with hearts in which the endocardium (containing the Purkinje fibers) was chemically ablated by applying a Lugol's iodine solution to the ventricular cavities (n=7). The ablation significantly reduced spontaneous activity in each phase of the protocol. Specifically, during acute regional ischemia, spontaneous activity was reduced by 80% ($p<0.005$); by 70% during low-flow reperfusion ($p<0.005$); and by 85% during full-flow reperfusion ($p<0.001$). Omission of blebbistatin, an electro-mechanical uncoupling agent, did not

change the diminishing effect of the ablation on spontaneous activity (n=3). Epicardial imaging showed that spontaneous ectopic beats were manifested as concentric epicardial breakthrough patterns, located near spatiotemporal gradients of NADH fluorescence. These data strongly suggest that in un-paced hearts from healthy rats that are perfused with Tyrode's solution, the main mechanism of spontaneous ectopic activity associated with either ischemia, low-flow or full-flow reperfusion is activation of local Purkinje fibers.

1324-Pos Board B168

Acute effects of Lipopolysaccharide on L-type Ca2+ channel currents and Transient Outward K+ channel currents in Rat Ventricular Myocytes

Dushon DeVere Riley[1,2], W. Jonathan Lederer[1,2].
[1]University of Maryland, Baltimore, Baltimore, MD, USA, [2]Institute of Molecular Cardiology, University of Maryland Biotechnology Institute, Baltimore, MD, USA.

Septic Shock has accounted for nearly 2 percent of all hospitalizations and has a mortality rate of 40-50%. It is a progression of sepsis, which is caused by bacterial infection in the blood and is characterized by a whole body inflammatory state known as systemic inflammatory response syndrome (SIRS). Cardiac dysfunction is one of the principal pathologies of sepsis and septic shock, along with other hemodynamic changes and dysfunction in multiple organs including the lungs and brain. While cardiac dysfunction is typically associated with the late stages of septicemia in clinical observations, animal models of septicemia have shown that cardiac dysfunction can occur well before late stages of sepsis and the induction of septic shock. Sepsis is caused by exposure to lipopolysaccharides (LPS), an endotoxin found in the outer membrane of gram-negative bacteria. LPS is a known Toll-like receptor 4 (TLR4) agonist which is associated with signaling cascades that lead to cellular inflammatory events. Studies have shown that short term exposure to LPS cause a significant increase in the amount of phosphorylated NF-κB in ARVCs. Using patch clamp single-cell electrophysiology we investigate acute effects of LPS exposure on membrane currents of adult rat ventricular cardiomyocytes (ARVC) *in vitro*. Given the necessity of L-type Ca^{2+} currents for proper myocardial function through excitation-contraction coupling (ECC) with links calcium membrane current with myocardial contraction, we investigate changes in L-type Ca^{2+} ($I_{Ca}{}^{2+}$). Due to its role in shaping the early phase of cardiac ventricular action potential we also investigate alterations of the transient outward Ca^{2+}-independent K^+ current (I_{TO}).

1325-Pos Board B169

Investigating Ion Channel Diseases With Dynamic Action Potential Clamp

Stefan A. Mann, Adam Hill, Jamie I. Vandenberg.
Victor Chang Cardiac Research Institute, Darlinghurst, Australia.

The number of ion channel mutations that are found in genetic screening of patients with cardiac arrhythmias is far outstripping our ability to functionally characterise all the mutations and assess the *in vivo* consequences of each mutant. Currently much time has to be invested in developing mathematical models of mutant ion channels in order to be able to predict their functional significance *in vivo*.

Recently the concept of a dynamic action potential clamp system was introduced that allows integration of electrophysiological recordings from mutant channels into *in silico* models of cellular systems. The current of interest is replaced in the model by current recorded from a mutant channel recorded using voltage-clamp electrophysiology, thereby bypassing the need to formulate a new mathematical model for the mutant ion channel. To simultaneously compute the action potential in the *in silico* model and record the ion current from a cell necessitates the use of a real time operating system so that the real cell can be clamped at the membrane potential of the virtual cell at all times (hence the term dynamic action potential clamp). The ionic current flow through the expressed channels would in turn contribute to changes in the membrane potential of the virtual cell model, so integrating the mutant channels into the virtual cell. We have developed a system where gating models are formulated using the graphical approach offered by Simulink / Matlab. Representation of the models in this way greatly simplifies the user interface compared to standard programming languages, making the system accessible to the less computer-savvy.

In the future we will use this system to study the effects of ion channel mutations on the cardiac function cardiac action potential.

1326-Pos Board B170

Estimating Contribution Of Individual Ionic Components To The Cardiac Pacemaker Potential

Yukiko Himeno[1], Chae Young Cha[1], Takao Shimayoshi[2], Yasuhiko Nakamura[1], Jian-Wu Wang[1], Akinori Noma[3,1], Nobuya Inagaki[1].
[1]Graduate School of Medicine, Kyoto University, Kyoto, Japan, [2]ASTEM Research Institute of Kyoto, Kyoto, Japan, [3]Faculty of Lifescience, Ritsumeikan University, Kusatsu, Japan.

Action potentials are generated by complicated interactions of various ionic channels and transporters through variations of membrane potential and/or the intracellular Ca^{2+} concentrations. It is still difficult to isolate the contribution of individual current from the secondary effect of modified channel activities. For example, if an ion channel is blocked, the pacemaker activity is drastically changed from the control time course. We have proposed a theoretical method to visualize the contribution of each current in the simulation study. Namely an instantaneous equilibrium potential, the lead potential (V_L), was calculated along the time course of pacemaker potential. V_L is given as,

$$V_L = (\Sigma G_X E_X - \Sigma I_{pump})/\Sigma G_X,$$

where G_X and E_X are whole cell membrane conductance and reversal potential for ion X. Contribution of a given current system is evaluated by comparing V_L obtained by fixing the gating parameter of a given current system with the control V_L. In the present study, the gating parameters are fixed during slow diastolic depolarization and action potential repolarization.

It is revealed that the initial phase of the slow diastolic depolarization is mostly attributable to deactivation of the rapid component of the delayed rectifier K^+ current (I_{Kr}). Then, the major inward currents, the hyperpolarization-activated current (I_f) and the sustained inward current (I_{st}) are activated to depolarize the membrane further to reach the threshold potential of the L-type Ca^{2+} channel current (I_{CaL}). Activation of I_{CaL} initiates generation of action potentials. Deactivation of I_{CaL} and activation of I_{Kr} and the slow component of the delayed rectifier K^+ current (I_{Ks}) occur during repolarizing phase. Contribution of I_{st} activation to the repolarization is much larger than that of I_f. Activation of I_f, I_{st}, I_{CaL} and I_{Ks} through phosphorylation during β1-adrenergic stimulation failed to modify each contribution dramatically.

1327-Pos Board B171

Extracellular Zinc Enhances Cardiomyocyte Relaxation Function in Diabetic Rats

Ting Yi, Zengyi Chen, Peter C. Van Buren, Bradley M. Palmer.
Unversity of Vermont, Burlington, VT, USA.

Diabetes mellitus (DM) leads to a cardiomyopathy in humans and rodent models. Interestingly, the chronic infusion of zinc in DM mice prevents the development of the cardiomyopathy. To elucidate the possible mechanism underlying this observation, we examined the effects of extracellular zinc ion (Zn^{2+}) on cardiomyocyte function in DM rats (n=5) compared to non-DM controls (Ctrl, n=5). Both the DM and Ctrl rats were hypothyroid, which assured similar upregulation of β-myosin heavy chain (β-MHC) in both populations. Isolated cardiomyocytes were electrically stimulated at 2, 4, 6 and 6.5 Hz, maintained at 35°C and exposed to 1.2 mM extracellular Ca^{2+}. Sarcomere shortening and relengthening dynamics were monitored using a video-based Fourier-transform technique. Without extracellular Zn^{2+} peak shortening as a fraction of diastolic sarcomere length was statistically greater ($P<0.05$) in the DM (6.56 ± 0.99 %; n = 16) compared to Ctrl (5.27 ± 1.91 %; n = 20) at 2 Hz, but not at higher frequencies. Time to 50% return to diastolic sarcomere length was not statistically different between the groups at every pacing frequency. Exposure to 12 μM extracellular Zn^{2+} significantly reduced ($P<0.001$) peak shortening in both the DM and Ctrl at all frequencies. There was a strong trend ($P = 0.070$) toward Zn^{2+} exposure significantly shortening the time to 50% return in the DM but not in the Ctrl, as revealed by repeated-measures ANOVA. Similar trends were found for time to peak shortening ($P=0.087$) and time to 10% return ($P=0.048$). These results suggest that extracellular Zn^{2+} inhibits cardiomyocyte contractile function independent of DM, perhaps by competing with intracellular Ca^{2+}, and enhances cardiomyocyte diastolic function in the DM. The enhanced sensitivity of the DM to the relaxing effects of Zn^{2+} may underlie the protective effects of Zn^{2+} against diabetic cardiomyopathy.

1328-Pos Board B172

Chronic Iron-overload Causes Sinus Bradycardia By Altering Electrical Activity In Sinoatrial Node Myocytes

Michael Sellan, Robert A. Rose, Carlo Cifelli, Scott P. Heximer, Peter H. Backx.
University of Toronto, Toronto, ON, Canada.

Treatment of blood disorders such as thalassemias require constant blood transfusions that cause iron-overload leading to iron-mediated cardiomyopathy, which is characterized by contractile dysfunction and electrical disturbances, especially bradyarrhythmias. In this study we explored the cellular mechanisms underlying iron-mediated bradycardia by examining the effects of chronic iron-overload (CIO; 0.6 mg/g iron-dextran 3 days/week for 4 weeks by IP injection) on heart rate and sinoatrial node (SAN) function in mice. As expected, heart rate (assessed with telemetry electrocardiograms), was lower ($p<0.001$) in CIO mice (509 ± 21 beats/min; $n = 5$) compared to controls (601 ± 12 beats/min; $n = 6$, dextrose injected). To examine intrinsic SAN function, heart rate was further studied in anesthetized mice following autonomic nervous system blockade with propranolol (10 mg/kg IP) and atropine (1 mg/kg IP), as