of functionalized force imaging, our result illustrates that activation of the integrin-fibronectin system is closely related to the VEGFR2 system via common mediators involved in signal transduction.

2982-Pos The hydroelastic curvature mechanism of Venus flytrap closing

Vladislav S. Markin[1], Emil Jovanov[2], Tejumade Adesina[3], Alexander G. Volkov[3]

[1] *University of Texas Southwestern Medical Center, Dallas, TX, USA,*
[2] *University of Alabama in Huntsville, Huntsville, AL, USA,*
[3] *Oakwood College, Huntsville, AL, USA.*

Board B285

The Venus flytrap captures insects with one of the most rapid movements in the plant kingdom. Here we present detailed experimental investigation of the trap closure by mechanical and electrical stimuli and the model of this mechanism, which provides accurate description of experimental data. The leaf is assumed to comprise two hydraulic layers at its inner and outer surfaces of the leaf, where pressure difference can be maintained. The minimum elastic energy of the leaf, including mean and Gaussian curvature, corresponds to the closed state. The open state is the energized configuration created by pressure difference. The opening of channels between two hydraulic reservoirs triggers the trap closing. Resulting flux of water removes the pressure holding the trap ajar and the system relaxes to its closed state with minimum energy. Trap closing by electrical stimulus obeys the all-or-none law: there is no reaction for under-threshold stimulus and the speed of closing does not depend on stimulus strength above threshold. We used uncouplers, blockers of ion channels and aquaporins to interrogate mechanisms of different phases of closing. The novel non-invasive charge-injection method together with physiologically active agents gives insight into different steps of signal transmission and responses in plant kingdom.

2983-Pos Electrical Response of Higher Plants to Induced Heat Stress

Ryan D. Lang, Maia I. Volkova, Albert J. Osei, Alexander G. Volkov

Oakwood College, Huntsville, AL, USA.

Board B286

Action potentials in higher plants are theorized as the information carriers in intercellular and intracellular communication in the presence of environmental stressors [1,2]. Among the most common stressors is heat stress. The response reactions of plant tissues and organs can be local or transmitted over long distances. Heat shock proteins, found in plant and animal cells, are partly responsible for the rapid response of plants to stress and the repair of plant tissue that has been damaged by stress through the activation of various pathways. In this work, the speeds of propagation of thermally induced action potentials in green plants are discussed. The speeds were found to be comparable to those occurring in various mammalian species. These rapid action potentials in green plants were recorded in real time using modern data acquisition techniques. According to our measurements, a single application of localized heat stress induces fast action potentials in *Aloe vera* (67 m/s). Electrical signals propagated along all leaves of the *Aloe vera* plants were studied. Possible pathways for electrical signal propagation in vascular plants are also discussed.

References

1. A. G. Volkov (Ed.) Plant Electrophysiology, Springer, Berlin, New York, 2006.
2. O. S. Ksenzhek, A. G. Volkov, Plant Energetics, Academic Press, San Diego, 1998.

Cardiac Electrophysiology

2984-Pos Remodeling of KCNE2 Subcellular Localization in Decompensated Heart Failure

Mansoureh Eghbali, Tomoaki Saito, Shuxun Ren, Yibin Wang, Ligia Toro, Enrico Stefani

Dept. Anesthesiology, Division of Molecular Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA.

Board B287

The molecular correlates of $I_{to,f}$ currents, Kv4.3, Kv4.2 and KChIP2 transcripts are downregulated in heart failure. KCNE2, a modulatory β-subunit, can associate in expression systems with Kv4.2/Kv4.3 channels potentiating current amplitude. We speculated that KCNE2 transcripts and/or expression might be modulated in pathological heart hypertrophy induced by pressure overload. The transaortic constriction (TAC) procedure was used to create pressure overload and heart failure (TAC-HF) in male mice. Real-time PCR showed that transcript levels of Kv4.3 (1±0.07 to 0.53±0.07), Kv4.2 (1±0.13 to 0.34±0.01) and KChIP2 (1±0.1 to 0.6±0.06) were downregulated in TAC-HF. On the other hand, KCNE2 transcripts were not significantly different between TAC-HF and CTRL. To gain insight on the potential KCNE2 association with Kv4.2/Kv4.3 channels and how this association may change in TAC-HF, cardiomyocytes from CTRL and TAC-HF were labeled with anti-KCNE2, -Kv4.2 and -Kv4.3 antibodies. Both Kv4.3 and Kv4.2 localizes mainly along the T-tubules with almost no labeling at the surface membrane, whereas KCNE2 was distributed both at the surface and tubular membranes. Kv4.3 and Kv4.2 subcellular localization did not change in TAC-HF, whereas KCNE2 completely disappeared from the T-tubules and only was distributed at the surface membrane. The fact that Kv4.3 and Kv4.2 distribution was similar in both TAC-HF and CTRL animals supports the view that the remodeling of KCNE2 in decompensated TAC is not the result of a disruption of the T-tubular system but it is a specific KCNE2 remodeling. We speculate that the reduction of Kv4.3 and Kv4.2

transcript levels in TAC-HF, as well as disappearance of KCNE2 from the T-tubules which in turn would reduce the association of KCNE2 with Kv4.3 and/or Kv4.2, may result in a further reduction of $I_{to,f}$ currents leading to the longer QT interval.

Supported by NIH and AHA.

2985-Pos The Heart as a Learning Machine

KrishnanJ [1], V.S. Chakravarthy[2]

[1] *Annamalai University, Annamalai Nagar, India,*
[2] *Indian Institute of Technology, Madras, Chennai, India.*

Board B288

Memory in the nervous system is essentially a network effect, resulting from activity-dependent synaptic modification in a network of neurons. Like the nervous system, the heart is a network of cardiac cells electrically coupled by gap junctions. The heart too has memory, termed cardiac memory. The phenomenon of cardiac memory refers to the property of cardiac tissue whereby the effect of an external electrical activation outlasts the duration of presentation of stimulus by a significant margin. We have earlier proposed that adaptation of gap junctions, as a function of membrane voltages of the cells that are coupled by the gap junctions, is related to cardiac memory. Using the proposed mechanism, we demonstrated memory effect using computational models of interacting cell pairs and also addressed the biological validity of the proposed mechanism of gap junctional adaptation. In the simulation studies, grid (30x30) of Noble cells, model of autorhythmic cardiac cells capable of spontaneous oscillations, is stimulated by an external current presented at the center of the grid. In normal situation a wave of depolarization sweeps from corner to corner in a periodic fashion, resembling the spread of depolarization in the real heart. A virtual Electrocardiogram (ECG) can be computed from the activity of the simulated grid. Using this grid of auto-rhythmic cardiac cell models the present work shows that prolonged external stimulus produces persistent changes in activation sequence, which may be seen in computed ECG. This studies on grid it is observed that the gap junction adaptation mechanism that helps to clear a brief and small magnitude disturbance retains effects of external input presented for a longer duration, which manifests as a memory.

2986-Pos Instant Cells With High And Constant Cell Quality: A Reliable Tool For Safety Pharmacology

Olaf Scheel, Corina Ehnert, Gesa Rascher-Eggstein, Thomas Knott

Cytocentrics AG, Rostock, Germany.

Board B289

Single cell based high throughput screening is an indispensable tool for compound testing in the pharmaceutical industry. Reliable data rest upon a high capacity cell culture providing the constant high quality of cells needed. We have developed a cell culture system which guarantees a high cell quality at a minimum amount of work and time. The frozen Instant Cells are ready to use in less than 15 minutes and kept in the Cell Reservoir, a bench-top storage device to preserve suspended cells in a non-clustered state and at a high vitality. We have adapted CHO-K1 and HEK 293 cells stably transfected with the human ether-a-go-go-related gene (hERG) potassium channel to the Instant Cells system.

Using patch clamp we show that our HEK hERG instant cells have the same characteristics in terms of electrophysiological and pharmacological properties compared to permanently cultured cells: >80 % sealed (>1 GΘ) and >60 % were stable for 15–25 min (>500 MΘ). The IC_{50} value for quinidine as well as the IV curves were not changed by freezing and thawing process. Quinidine IC_{50} values obtained with the planar patch clamp automate Cytopatch™ were in good agreement with data obtained with a manual patch clamp setup using the Dynaflow™ system (Cellectricon, Sweden).

This proves that the Instant Cells system is well suited to investigate hERG ion channel pharmacology using different patch clamp approaches. The frozen batches are routinely quality controlled and therefore the Instant Cells provide a reliable tool for using them for hERG safety screening ensuring a constant high cell quality overcoming quality changes of the running cell culture.

2987-Pos Expression Of The ΔY475 Lqt2-linked Mutation Current In Neonatal Mouse Cardiomyocytes

Eric C. Lin, Jing Wang, Brain P. Delisle, Katherine M. Holzem, Evi Lim, Craig T. January

UW-Madison, Madison, WI, USA.

Board B290

Mutations in the *human ether-a-go-go related gene* (*hERG*) are linked to one form of long QT syndrome (LQT2). *hERG* encodes the pore-forming α-subunits that underlie the rapidly activating delayed rectifier K^+ current (I_{Kr}) in the heart. Heterologously expressed WT-hERG channels, such as in HEK293 cells, demonstrate biophysical and pharmacological properties similar to native I_{Kr}. Little is known about the properties of LQT2-channels in native cardiomyocyte systems. In this study, we heterologously expressed WT- and ΔY475-hERG, a mutation that exhibits altered channel kinetics, in HEK293 cells or in neonatal mouse cardiomyocytes. Electrophysiological analysis of these channels used the whole cell patch clamp technique. When fit with the Boltzmann equation, the voltage dependence of activation for WT- and ΔY475-hERG showed $V_{1/2}$ values of −11.3±0.3 and −30.0±0.3 mV with slope factors of 6.9±0.2 and 6.1±0.2 in HEK293 cells (n=4), and −20.3±0.4 and −30.1±0.3 mV with slope factors of 5.8±0.3 and 5.3±0.3 in the neonatal cardiomyocytes (n=4), respectively. In HEK293 cells, the ΔY475-hERG deactivation rate at −50 mV (τ_{fast} =97.3±4.3 ms, τ_{slow} =400.0±24.9 ms) was accelerated compared to WT-hERG (τ_{fast} =586.7±47.7 ms, τ_{slow} =3793.1±333.1 ms). In the neonatal carydiomyocytes, the ΔY475-hERG deactivation rate (τ_{fast} =64.8±9.4 ms, τ_{slow} =266.3±53.4 ms) was also accelerated compared to WT-hERG (τ_{fast}= 273.9±17.5 ms, τ_{slow} =1740.7±163.9 ms). In conclusion, our data show that the WT- and LQT2-channels can

be studied in neonatal mouse cardiomycoytes. These data show some quantitative differences between WT- and ΔY475-hERG channels studied in HEK293 cells and neonatal mouse cardiomyocytes, however, the differences are small in magnitude.

2988-Pos Gender and Estrogen Determines Outward Potassium Current Densities in Mouse Ventricular

Myocytes Tomoaki Saito, Andrea Ciobotaru, Ligia Toro, Enrico Stefani, Mansoureh Eghbali

Dept. Anesthesiology, Division of Molecular Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA.

Board B291

Gender studies on fast-transient outward K^+ currents ($I_{to,f}$) and ultra-rapid delayed rectifier K^+ currents ($I_{K,slow}$) have yielded different results possibly due to missing information on the females estral stage. We revisited gender differences in K^+ currents in right ventricle cardiomyocytes taking into consideration the female estral cycle, where in diestrus2 estrogen is relatively low (~15 pg/ml) as in estrus, though estrus is primed by the estrogen surge hours earlier in proestrus (~60 pg/ml). Peak total K^+ current (I_{total}) densities (pA/pF, at +40 mV) were much higher in males (48.6±3.0) than in females at estrus (27.2±2.3), and intermediate at diestrus2 (39.1±3.4). Underlying this change, $I_{K,slow}$ and $I_{to,f}$ were higher in males than females at estrus and intermediate at diestrus2 ($I_{K,slow}$: male 24.0±1.8, estrus 14.1±0.9, diestrus2 19.3±1.9; $I_{to,f}$: male 26.8±1.9, estrus 14.9±1.6, diestrus2 22.1±2.1). Estrogen treatment of ovariectomized mice decreased I_{total} (46.4±3.0 to 28.4±1.6), with a robust inhibition of $I_{to,f}$ (26.6±1.6 to 12.8±1.0) and a lower $I_{K,slow}$ (22.2±1.6 to 17.2±1.4). Inward K^+ currents remained unaffected in all conditions. Action potential durations were significantly longer in estrus (4.5±0.8 ms at 0 mV and 32.9±5.9 at −60 mV) when compared to male (2.61±0.24 at 0 mV and 17.5±2.5 at −60 mV) and diestrus2 (2.62±0.13 ms at 0 mV and 17.8±1.2 at −60 mV). To gain insight into the mechanism(s) of gender differences, transcript levels of the molecualr correlates of $I_{to,f}$ (Kv4.3, Kv4.2 and KChIP2) and $I_{K,slow}$ (Kv1.5) were quantified in right ventricle. Kv4.3 and Kv1.5, but not Kv4.2 and KChIP2 transcripts were significantly lower in estrus than in diestrus2 and male supporting the role of estrogen in determining gender differences.

Supported by NIH and AHA.

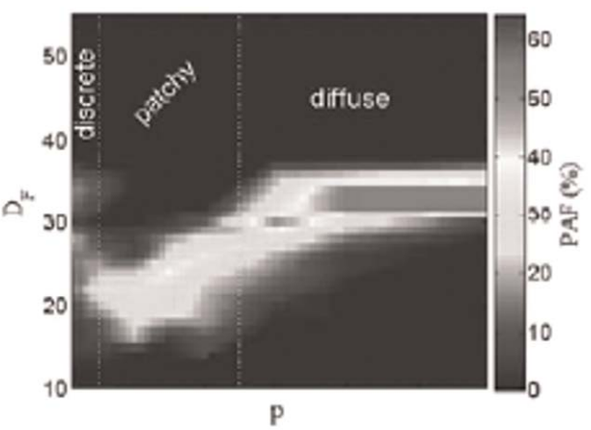
2989-Pos Interactions Between Atrial Fibrosis Density And Pattern In The Determination Of Atrial Fibrillation Probability: Insights From A Mathematical Model

Philippe Comtois[1,2], Stanley Nattel[1,3]

[1] *Montreal Heart Institute, Montreal, QC, Canada,*
[2] *McGill University, Montreal, QC, Canada,*
[3] *University of Montreal, Montreal, QC, Canada.*

Board B292

This study focuses on initiation of atrial fibrillation(AF) in the presence of fibrosis with different spatial structures. Method: The model is a two-dimensional cellular substrate with realistic atrial ionic currents and coupling. Fibrosis is mimicked by holes with the structure determined by a stochastic model controlled by pattern(p) and density(DF) parameters. The percent of cases with AF initiation (PAF) by burst electrical pacing is calculated with a Monte-Carlo approach(40 simulations per p and DF). Results: Diffuse fibrosis caused homogeneous slowing and sustained AF activity occurred over a small range of DF; PAF was very high in this range(Figure). As fibrosis became patchier, the mechanism of AF moved from functionally based towards physically anchored generators. As fibrosis became increasingly discrete, the range of DF over which AF could be sustained became larger, but maximum PAF decreased (Figure). Fibrosis promoted AF by impeding conduction and stabilizing spiral wave generators. Conclusion: Fibrosis pattern is an important determinant of AF mechanism and DF dependency. A combination of small and large fibrotic clusters might favor AF by decreasing the minimum density of fibrosis needed to initiate persistent AF.



2990-Pos Differential Effects of Timolol and Propranolol on Heart Function During Maturation

ERKAN TUNCAY, Hakan Gurdal, Belma Turan

health sciences, ankara, Turkey.

Board B293

β-adrenergic blockers are one of the most frequently prescribed drugs for the treatment of cardiovascular dysfunction showing differential effects either short or long terms uses. This study was designed to determine the effect of chronic treatment with beta adrenergic blockers on the electrical and mechanical activity of the heart during maturation in rat. Three month old male rats were treated (intragastrically) with two different non-selective beta ad-

renergic blockers; propranolol (25 mg/kg/day, n=17) or timolol (5 mg/kg/day, n=16) until the ages of 6 months. Hemodynamic and intracellular action potential parameters were determined in heart from 3 mo and 6 mo old beta adrenergic blockers treated and untreated control rats. Left ventricular developed pressure (LVDP) was significantly depressed in 6 mo old rats (30%, n=8) compared to those of 3 mo old rats (n=8). Timolol but not propranolol restored this depression to the level of those of 3 mo old rats. In addition, two late repolarization phases of action potential duration ($APD_{75,\ 90}$) were markedly prolonged (55% and 56%, respectively) in 6 mo old rats compared to those of 3 mo old rats. Timolol treatment completely restored the prolongation both in APD_{75} and $APD_{90,}$ while this restoration was partial with propranolol treatment in 6 mo old rats. On the other hand, resting membrane potential and peak depolarization of action potentials did not change by treatment with timolol or propranolol in 6 mo old rats. Our results demonstrated that despite the presence of differences in the effect of beta adrenergic blockers, treatment with these agents have some restoring effect on the age dependent changes in hearts of male rats during maturation.

Supported by projects TUBITAK-PIA-10 & Ankara Univ BAP20060809233

2991-Pos Subtype Specific β Adrenergic Control of Spontaneous Firing Rate and I_f in Murine Sinoatrial Myocytes

Cathy Proenza, Zhandi Liao

University of Connecticut, Storrs, CT, USA.

Board B294

The sympathetic nervous system accelerates heart rate by activating β adrenergic receptors (βARs) on sinoatrial myocytes. Hyperpolarization activated cyclic nucleotide sensitive (HCN) channels produce the cardiac I_f current and are thought to be among the effectors for this positive chronotropic effect. β_1 and β_2ARs are the predominant adrenoreceptor subtypes in cardiac tissue. Studies in cultured neonatal myocytes from knockout mice and in murine embryonic stem cell-derived cardiomyocytes suggest that β_1ARs may mediate almost all adrenergic control of heart rate in the mouse. However, it is not known whether this is true in fully-differentiated sinoatrial myocytes from adult mice. Moreover, the βAR subtype-specific control of I_f has not been described in murine sinoatrial cells. Here we have used subtype-specific blockers to identify the βAR subtype (s) involved in control of firing frequency and I_f in the mouse heart. Spontaneous action potentials were recorded from cells current-clamped in the cell-attached configuration. The non-specific β agonist isoproterenol (ISO, 1 μM) increased the firing frequency by ~40%, and this increase was unaffected by the presence of the β_2-specific antagonist, ICI-118551 (ICI, 1 μM). However, the β_1-specific antagonist, CGP20712A (CGP, 3 μM), reversibly abolished the ability of ISO to increase the firing rate. These results indicate that β_1ARs control firing rate in adult sinoatrial cells, and thus predict that β_1ARs should control I_f in these cells. To test this prediction, I_f was recorded from sinoatrial cells voltage-clamped in the whole cell configuration. Preliminary data suggest that the ability of ISO to shift the midpoint voltage of activation for I_f was unaffected by ICI, but was blocked by CPG. These observations are consistent with a role for HCN channels in adrenergic control of heart rate in the mouse.

2992-Pos Sex Differences In Ventricular Repolarization In Guinea-pig Hearts

Andrew F. James, Zhan Gao, Stephanie CM Choisy, Lesley A. Arberry, Jules C. Hancox

University of Bristol, Bristol, United Kingdom.

Board B295

Women have a higher incidence of drug-induced torsades de pointes than do men. The underlying mechanisms remain unclear but are likely to involve modulation of ventricular repolarization by the gonadal steroids. The female guinea-pig has been suggested to be a suitable model for the investigation of the involvement of ovarian steroids in ventricular repolarization. Age-matched (17–19 weeks) male and female guinea-pigs on different days of the estrus cycle (day 0, days 4–5 and days 13–14) were killed; the hearts excised and mounted on a Langendorff apparatus. Serum estradiol-17beta and progesterone concentrations were measured by radioimmunoassay. The right atrium and sinoatrial node were removed and hearts paced at cycle lengths (CL) of 150-375 ms. Monophasic action potentials were recorded from the epicardial surface of the left ventricle. The electrocardiogram was also recorded. The effects of quinidine (1–3 micromol/l) were examined at CL=375ms. Serum estradiol-17beta levels were maximal in females on day 0 (20.1±3.5pg/ml, n=7) and minimal on days 13–14 of the cycle (7.6±2.9pg/ml, n=5, $P<0.05$). Day 0 females showed significantly longer QTpk intervals than either males or females on days 13–14 over the range of pacing CL. In contrast, the differences in QTend were less marked, indicating sex differences in Tpk-Tend interval. Differences between the groups in action potential duration at 90% repolarization (APD90) over the range of CL used reflected those in QTpk interval. Quinidine-induced prolongations of QT intervals and APD90 were maximal in females on day 0 and minimal on days 13–14 of the cycle, prolongation of QTpk correlating closely with serum [estradiol-17β] (r2=0.995) but not progesterone (r2=0.1334). These data are consistent with a pro-arrhythmic effect of estradiol-17beta on ventricular repolarization.

Financial support from the British Heart Foundation (PG/03/121) is gratefully acknowledged.

2993-Pos Beneficial Effects of Chronic Treatment with Beta-adrenergic Blockers on Diabetic Cardiomyopathy

Erkan Tuncay[1], Hakan Gurdal[1], Belma Turan[2]

[1] *Ankara University Faculty of Medicine, Ankara, Turkey,*
[2] *Ankara University School of Medicine, Ankara, Turkey.*

Board B296

Cardiovascular diseases are major causes of mortality in patient population and β-adrenergic blockers are one of the most frequently prescribed cardiovascular drugs. Diabetes mellitus is a major risk

factor for multiple cardiovascular complications which can be characterized with left ventricular systolic dysfunction. Although there are some significant differences in the pharmacological actions of these agents on heart, their clinical effects are generally predictable. In this study, we examined the long-term effects of non-selective β-adrenoceptor blockers, propranolol (25 mg/kg/day, n=10)- or timolol (5 mg/kg/day, n=10) administrations (intragastrically, 3 months), on hemodynamic and intracellular action potential parameters of heart in adult diabetic male rats. Left ventricular developed pressure (LVDP) and the rates of changes of developed pressure (±dP/dt) were significantly depressed in 6 mo old diabetic rats compared to those of 6 mo old normal control rats (n=16). Timolol but not propranolol restored these depressions to the levels of those of the controls. Two late-repolarization phases of action potential duration (APD_{75} and APD_{90}) were significantly prolonged in 6 mo old diabetic heart compared to those of the controls. Timolol treatment significantly restored these prolongations in the diabetic hearts while propranolol treatment restored only prolongation in APD_{90}. On the other hand, resting membrane potential and peak depolarization of action potentials did not change by treatment with timolol or propranolol in 6 mo old diabetic rats. Our results showed that despite the presence of differences in the effect of beta adrenergic blockers, treatment with these agents have some beneficial effects on the dysfunction of diabetic male hearts.

Supported by projects: TUBITAK-PIA-10 & Ankara Univ BAP20060809233

2994-Pos Arrhythmia Studies Using Computational Model Of Ventricular Tissue

MalathiR , M.R.S. Reddy

Department of Applied Mechanics, Indian Institute of Technology, Chennai, India

Board B297

Cardiac arrhythmia is mainly due to failure in the coordination among contraction of cardiac muscles. Among several types of arrhythmias, this work focused on ventricular arrhythmia, because it is more dangerous than others. With the help of mathematical model of ventricle cell, the role of sodium and calcium ion on single cell, 1D array of cells and 2D grid of cells in establishing arrhythmia are studied. Three sets of simulation have been done. First one showed the effect of variation of Sodium ion concentration on single cell. It is observed that deviation from nominal value of sodium ion concentration shortens the action potential duration (APD). After understanding single cell behavior, our next sets of simulation study concentrated on cell pair and 1D array of cells. Both in cell pair as well 1D array of cells, delay in action potential propagation is observed due to variations in sodium ion concentration. In our last study, a grid of 60x60 cells is considered. The cells in the grid are connected via resistive connection, resembles gap junction in real cardiac electrophysiology. In the grid study at first normal activation pattern is observed. Then external stimulus is presented and resulted in generation of spiral waves in the grid. Also simulation studies have performed on the effect of spiral wave due to the variation of sodium ion concentration. It is believed that our studies from cellular level to tissue level makes to understand the causes of arrhythmia due to variation in ionic parameters. This ionic parameter changes in real environment it is due to the effect of drugs as well changes in dynamics of cell parameters.

2995-Pos CCD-based And 2P Confocal Measurements Of Electrical Alternans On The Epicardial Surface Of Isolated Perfused Rabbit Left Ventricle Using Voltage Sensitive Dyes

Rachel C. Myles, Ole J. Kemi, Francis L. Burton, Stuart M. Cobbe, Godfrey L. Smith

University of Glasgow, Glasgow, United Kingdom.

Board B298

Regions of myocardium exhibiting alternating action potential durations are thought to predispose to re-entrant ventricular arrhythmia. This phenomenon, termed repolarisation alternans, was studied in an isolated rabbit left ventricular wedge preparation at 30°C. Epicardial electrical activity was monitored using voltage sensitive dyes. In one set of experiments a CCD-camera detection system (single pixel dimension $326um^2$) was used to record optical action potentials. At a pacing cycle length (PCL) of 350ms, optical action potentials showed no alternating characteristics. The rise time of the signal (Trise, time from 10% to 90% of the signal) was 26±4ms and action potential duration at 75% of repolarisation (APD_{75}) was 155±4ms (n=4). Progressive reduction in PCL produced alternans of both action potential duration and amplitude. The mean change in alternate APD_{75} was 13±5% and in action potential amplitude was 35±12% (mean PCL 148±5ms). To investigate this further, optical action potentials were recorded from single cells within the wedge preparation using 2-photon excitation (930nm) at a depth of ~50μm from the epicardial surface with resolution of ~$1\mu m^2$ and a Z axis resolution of ~ 2μm. At a PCL of 350ms, APD_{75} was 185±3ms and Trise was 11±1ms (n=4). At the minimum PCL supported (160–170ms), some regions of myocardium displayed 2:1 block while in adjacent regions 1:1 coupling was observed. However, no significant changes in alternate action potential duration (8±10%) or amplitude (2±2%) were evident. These data suggest that rapid pacing and low temperature induce a heterogeneous response in rabbit myocardium with some regions displaying intermittent block, while others respond normally to each stimulus. This heterogeneous behaviour may underlie the alternating changes in optical action potential amplitude recorded from larger areas of myocardium.

2996-Pos Antiarrhythmic Effect of Rotigaptide on the Gating of Ventricular Gap Junctions

Xianming Lin[1], Christian Zemlin[1], Torben Peitersen[2], Neils-Henrik Holstein-Rathlou[2], Jorgen Petersen[3], James Hennan[4], Richard D. Veenstra[1]

[1] *SUNY Upstate Medical University, Syracuse, NY, USA,*
[2] *University of Copenhagen, Copenhagen, Denmark,*
[3] *Zealand Pharma A/S, Glostrup, Denmark,*
[4] *Wyeth Research, Collegeville, PA, USA.*

Board B299

Rotigaptide is a novel antiarrhythmic peptide that exerts its effects on reentrant forms of ventricular tachycardias by improving electrical cell-cell coupling. To further elucidate the possible mechanisms of action of rotigaptide on cardiac gap junctions, we investigated the effects of rotigaptide treatment on the gating of ventricular gap junctions using the dual whole cell action potential voltage clamp method. Pretreatment with $\leq$ 100 nM rotigaptide increased the steady state minimum gap junction conductance (G_{min}) maximally by 10% without alteration of the transjunctional voltage (V_j) sensitivity of inactivation. The first order kinetics of both fast and slow V_j-dependent inactivation were slowed in a concentration-dependent manner without alteration of their similar V_j-dependence. A bell-shaped dose-response curve was observed with concentrations $>$ 100 nM rotigaptide producing a decreasing response. Rotigaptide did not alter the recovery of junctional conductance (G_j) that occurs from with decreasing V_j from inactivating potentials. The effects of 0 – 100 nM rotigaptide on ventricular G_j during the cardiac action potential was accurately modeled by accounting for the concentration-dependent effects on the inactivation kinetics. Computer simulations demonstrate that inactivation of ventricular gap junctions slows conduction more than partial uncoupling alone and that rotigaptide can reverse this effect. Electrical uncoupling promotes slow, discontinuous action potential propagation, unidirectional conduction block, and the formation of reentrant arrhythmias. We propose that the reduction of ventricular G_j inactivation by rotigaptide is one mechanism for the antiarrhythmic properties of this peptide.

Supported by NSF, NBCR, California Institute for Telecommunication and Information Technology

2997-Pos Optimizing Cardiac Excitation-Metabolic Model By Using Parallel Grid Computing

Saleh Amirriazi[1], Stephany Chang[1], Tom Peachey[2], David Abramson[2], Anushka Michailova[1]

[1] *University of California San Diego, La Jolla, CA, USA,*
[2] *Monash University, Clayton, Victoria, Australia.*

Board B300

We have extended the Shannon-Bers model in order to investigate excitation- contraction coupling in rabbit epicardial and endocardial ventricular myocytes [1]. We couple cytosolic metabolism to the cell electrical activity and include rate expressions for Mg2+-nucleotide regulation of ATP-sensitive K+ channel, L-type Ca2+ channel, sarcolemmal and sarcoplasmic Ca2+-ATPases, and Na+/K + pump [2]. The work was performed with a distributed parameter optimization tool (Nimrod/O) to search for stable model solutions [3]. The Nimrod experiment involved validation of the updated model by varying

(a) input current parameters to stabilize the normal epi- and endocardial Ca2+ transients for time interval of 3 min at 0.5Hz,
(b) input metabolic constants to fit the predicted normal ionic currents to be as close as possible to the experimentally suggested.

The results suggest that the distribution of ATP-sensitive K+ channels might be important mechanism regulating the excitation-contraction coupling during ischemia.

References

[1]. Shannon et al., (2004) Biophysical Journal *87*: 3351–3371.
[2]. Michailova at al., (2007) AJP Cell Physiology *293*: 542–557.
[3]. Nimrod Portal http://www.csse.monash.edu.au/~davida/nimrod/portal.htm

2998-Pos Unmasking the Substrate for Phase-2 Reentry Using Mathematical Modeling

Anat Maoz[1], Trine Krogh-Madsen[2], David J. Christini[2,3]

[1] *Weill Cornell Medical College/Cornell University/Sloan Kettering Institute Tri-institutional Program in Computational Biology and Medicine, New York, NY, USA,*
[2] *Division of Cardiology, Weill Cornell Medical College, New York, NY, USA,*
[3] *Department of Physiology, Biophysics and Systems Biology, New York, NY, USA.*

Board B301

In recent years a dynamic known as phase-2 reentry (P2R) has been recognized as a mechanism for triggering lethal arrhythmias. In particular, P2R may underlie arrhythmogenesis in individuals with structurally normal hearts who have genetic cardiac diseases, such as Brugada syndrome. P2R occurs when adjacent cells undergo dramatic action-potential (AP) shortening from a normal notch-and-dome morphology to a loss-of-dome morphology. Local re-excitation ensues when ionic current during the AP dome stage propagates from depolarized sites to hyperpolarized loss-of-dome sites. It is thought that P2R could degenerate into ventricular fibrillation and cause sudden cardiac death.

A cellular mechanism for P2R has been proposed in which the factor that leads to "loss of dome" is the rebalancing of the currents available at the end of phase-1 of the AP. However, the necessary conditions and spatial mechanism for P2R are still relatively unknown.

In this study, we used multiple mathematical ionic cardiac models to investigate this mechanism. Specifically, we investigated emergence of discontinuous repolarization and P2R initiation in epicardial cables with continuous, uninterrupted ionic Ito expression-level gradients. We hypothesized that with such gradients, adjacency of loss-of-dome and notch-and-dome cells is not sufficient for P2R occurrence due to the smoothing effects of electrotonic currents. We found that the main factor facilitating P2R is not the presence of these two different morphologies in adjacent cells, but rather bistable AP morphology within a significant subset of cells. With such bistability, cells can switch intermittently between notch-and-dome to loss-of-dome morphologies, which can trigger P2R. These

findings suggest that such switching behavior may be one of the bases for P2R development in cardiac tissue.

2999-Pos Modeling Transmural Heterogeneity of Repolarization and Ca^{2+} Handling in Mouse Ventricle

Vladimir E. Bondarenko, Randall L. Rasmusson

University at Buffalo, SUNY, Buffalo, NY, USA.

Board B302

The mouse heart contains a variety of cardiac myocyte types that generate a diversity of action potentials (AP) with different shapes, durations, and different levels of ion channel and transporter expression. Recent evidence supports the notion that cells from the epicardial and endocardial regions of the mouse ventricle have altered calcium handling properties as well as different potassium current compositions. Both calcium handling and potassium current expression have been implicated in differences in the transmural heterogeneity of repolarization. Our computer model simulates the following differences between epicardial and endocardial myocytes:

1. action potential duration is longer in endocardial and shorter in epicardial myocytes;
2. diastolic and systolic $[Ca^{2+}]_i$ and $[Ca^{2+}]_i$ transients are higher in paced endocardial and lower in epicardial myocytes;
3. Ca^{2+} release rate is about two times larger in endocardial than in epicardial myocytes;
4. Na^+/Ca^{2+} exchanger rate is greater in epicardial than in endocardial myocytes.

To investigate predicted relative contributions of action potential waveform versus Ca^{2+} release mechanisms to transmural heterogeneity of Ca^{2+} transient behavior, we simulated action potential clamp of epicardial myocyte models with endocardial APs and endocardial myocyte models with epicardial APs. Our data indicate that the difference in Ca^{2+} release rate is the major contributing factor to heterogeneity of Ca^{2+} transients in the mouse ventricle. Cellular differences altered the stability of predicted Ca^{2+} transients and action potential duration. Endocardial cells showed a lower threshold pacing rate for stability of AP generation than epicardial myocytes. However, epicardial cells showed a more complex pattern of AP duration at higher frequencies. Finally, simulation of AP propagation in 2D model tissue demonstrated transmural heterogeneity of both repolarization and Ca^{2+} handling.

3000-Pos A Single-Cell Model of Phase-Driven Control of Ventricular Fibrillation Frequency

Krzysztof R. Grzeda, Justus M. B. Anumonwo, Ryan O'Connell, Jose Jalife

SUNY Upstate Medical University, Syracuse, NY, USA.

Board B303

Introduction: The mechanisms controlling reentry rotation frequency (RRF) in ventricular fibrillation (VF) are poorly understood. It has been shown that Ba^{2+}, which at <=50 μmol/L selectively blocks the inward rectifier potassium current, I_{K1}, slows RRF in the intact guinea pig (GP) heart in a dose-dependent manner (Warren et al, 2003).

Hypothesis: Control of the RRF by I_{K1} blockade is phase-driven, i.e. the phase difference between transmembrane current and voltage remains constant at varying barium concentrations.

Methods: GP left ventricular myocytes were isolated from the left ventricle using retrograde perfusion method. To approach conditions prevailing near the reentry core in the intact heart, we applied 5 – 50 Hz sinusoidal voltages of 15 mV amplitude together with a −35 mV DC offset. The voltage values covered the I_{K1} negative slope region and frequencies covered the range of VF frequency in the GP heart. From the current and voltage signals the components of the fundamental frequency were extracted and represented as complex numbers. The admittance phase (i.e., the phase difference between current and voltage) was plotted vs. frequency in control conditions and at 10 or 50 μmol/L Ba^{2+}.

Results: The admittance phase vs. frequency plot was shifted to the left by 14.14±5.71 Hz (n=14) at 10 μmol/L Ba^{2+} and by 18.51±4.00 Hz (n=10) at 50 μmol/L Ba^{2+}, $p<0.05$. The values matched the Ba^{2+}-induced reduction of VF frequency observed previously in GP heart.

Conclusions: We have developed a simplified single cell model of RRF control. The results show that while RRF changes as a result of I_{K1} blockade, the phase difference between transmembrane current and transmembrane voltage remained constant, as hypothesized. Thus our single cell approach allows us to quantitatively predict the change of VF frequency resulting from I_{K1} blockade.

3001-Pos Heuristic Tuning of Myocyte Model Parameters for Cardiac Arrhythmia Research

Stephen A. Gaeta[1], David Christini[2,3]

[1] *Weill Cornell/Rockefeller/Sloan-Kettering Tri-Institutional MD-PhD Program, New York, NY, USA,*

[2] *Division of Cardiology, Weill Cornell Medical College, New York, NY, USA,*

[3] *Department of Physiology, Biophysics, and Systems Biology, Weill Cornell Medical College, New York, NY, USA.*

Board B304

The traditional, piecemeal approach to the creation of cardiac myocyte mathematical models is inadequate for many problems in arrhythmia research. Whole-cell models are usually created as collections of individual ion channel descriptions, each of which is tuned in isolation. This method is problematic, in that models are used to research whole-cell, or even whole-organ dynamics, and often in contexts far from the experiments to which the channel equations were initially fit. These models thus often do not faithfully capture many of the dynamical properties responsible for arrhythmogenesis, which occur as a result of interactions between cell

components, and usually at excitation patterns other than those of the experimental data used to tune the model. Using a genetic algorithm, we have constrained parameters of the existing Shiferaw-Fox myocyte model to better fit our own perforated-patch voltage and intracellular calcium fluorescence data. Moreover, we have fit parameters to data from dynamic pacing protocols capturing a wide range of myocyte behavior, allowing better constraint and a more robust model. This technique is a means of effectively and appropriately creating accurate representations of individual cardiac myocytes, which are able to faithfully reproduce cardiac dynamics in contexts relevant to arrhythmia research.

3002-Pos Measuring the Slopes of Restitution Curves in Cardiac Tissue with a Random Pacing Protocol

Jan P. Kucera, Enno de Lange

University of Bern, Bern, Switzerland.

Board B305

In cardiac tissue, conduction velocity (CV) and action potential duration (APD) tightly depend on the previous diastolic interval (DI). This dependence, called restitution, is an important determinant of the stability of cardiac excitation. Restitution can be targeted by antiarrhythmic drugs and the slopes of restitution curves represent a key factor in arrhythmogenesis. We explored the possibility to determine the slopes of the S1-S2 APD and CV restitution curves (α and γ, respectively) using CV measurements during random pacing. Based on a linearization of CV and APD restitution relationships, we derived an equation relating the CV of a given action potential (AP) with the CV of the previous one and the last cycle length (CL). This equation represents an autoregressive-moving-average (ARMA) model, with CL as input series, CV as output series, α as the autoregressive coefficient and γ as the moving average coefficient. Identification of the ARMA model based on a known CL series and a corresponding CV series may thus permit to determine both α and γ.

This hypothesis was tested in simulations of conduction in a Luo-Rudy model cell strand. The strand was paced at given CLs and the slopes α and γ were first determined using a conventional S1-S2 protocol. CL was then perturbed with normally-distributed beat-to-beat variations with zero mean and a standard deviation of 5 ms for 30 consecutive APs. From the CL and CV series, α and γ were then computed via ARMA model fitting. The computed values were equivalent within 95% confidence intervals to the values obtained with the S1-S2 protocol.

This result therefore indicates that pacing at randomly varying intervals represents a powerful strategy to obtain information about both APD and CV restitution without the necessity of measuring APD.

3003-Pos Computer Model of the Neonatal Mouse Action Potential

Linda J. Wang, Eric A. Sobie

Mt. Sinai School of Medicine, New York, NY, USA.

Board B306

Most therapies for heart disease are based on our understanding of the function of the adult heart. The dramatic differences in action potential(AP) shape between neonatal and adult cardiac myocytes, however, indicate that a different set of molecular interactions operates in the hearts of newborns, and different therapies may therefore be appropriate for these patients. Computational modeling is a useful method for synthesizing data obtained from multiple sources to determine how changes in the behavior of multiple individual entities may lead to changes in function. However, the use of this technique to study the function of neonatal cardiac myocytes has been limited. We created a mathematical model of the AP of the neonatal mouse myocyte by beginning with a model of the adult cell and modifying the densities and/or formulations of ion transport mechanisms based on experimental data obtained in neonatal cells. The new model reproduces the AP shape characteristic of neonatal mouse cells, with a brief plateau phase and longer duration (APD_{80}=59.2 ms) compared with the adult (APD_{80}=12.5 ms). The simulation results are broadly consistent with a wide range of experimental data, including:

1. decreased density, and altered inactivation properties, of transient outward K^+ currents,
2. increased delayed rectifier K^+ currents,
3. Ca^{2+} entry through T-type as well as L-type Ca^{2+} channels,
4. increased Ca^{2+} influx through Na^+-Ca^{2+} exchange,
5. decreased cytosolic Ca^{2+} buffering, and
6. Ca^{2+} transients that rely primarily on sarcolemmal Ca^{2+} entry rather than SR Ca^{2+} release.

Simulations performed under AP clamp conditions suggest that the longer AP observed in neonatal cells is critical for maintaining Ca^{2+} transient amplitude in these cells. This model can be used in the future to generate novel predictions and to gain a better quantitative understanding of differences between neonatal and adult physiology.

3004-Pos Supernormal Conduction of Premature Impulses Potentiates Alternans in Cardiac Tissue

Enno de Lange, Jan P. Kucera

University of Bern, Bern, Switzerland.

Board B307

Alternans is a cardiac proarrhythmic mechanism that can result from a steep slope of the action potential duration (APD) restitution curve. The contribution of conduction velocity (CV) restitution to alternans is however still largely unknown. We investigated whether supernormal conduction of premature impulses, characterized by a negative CV restitution slope, may render conduction unstable and potentiate alternans.

Using microelectrode arrays, we measured CV in patterned strands of neonatal rat ventricular myocytes. CV restitution was assessed using a standard S1-S2 protocol and a pacing protocol incorporating random Gaussian variations of cycle length (CL) around the basic CL. This random protocol permits the analysis of the stability of conduction on a beat-to-beat basis using techniques

from dynamical systems theory. Supernormal conduction was induced by reducing $[K^+]_o$ to 2.0 mmol/L. In parallel, the influence of CV restitution on conduction stability was investigated using a coupled maps mathematical model.

Under control conditions, CV decreased in the patterned strands with increasing impulse prematurity and the CV restitution curve was characterized by a positive slope. Therefore, with increasing distance from the stimulation site, the slower conduction of premature impulses attenuated the CL variations. Spectral analysis revealed that this attenuation was maximal at a frequency of 0.5 $beat^{-1}$. In contrast, when $[K^+]_o$ was reduced, CV increased with increasing impulse prematurity and the slope of the CV restitution curve was negative. Consequently, the faster conduction of premature impulses amplified the CL variations along the propagation pathway, indicating that supernormal conduction is intrinsically unstable. This amplification was maximal at a frequency of 0.5 $beat^{-1}$, corresponding to alternans. The coupled maps model indicated that this amplification occurs even in the absence of APD restitution.

We conclude that supernormal conduction of premature impulses represents by itself a mechanism of alternans.

3005-Pos Simplified Parameter Sensitivity Analysis in Computational Models of Cellular Electrophysiology

Eric A. Sobie

Mount Sinai School of Medicine, New York, NY, USA.

Board B308

Computational models of electrical activity and calcium signaling in myocytes have long been important tools for understanding cardiac physiology. Each year, additional models are published, with the number of processes considered continually increasing. A drawback of the complexity of contemporary models, however, is that the changes in model outputs caused by alterations in parameters are often not well-understood, since parameter sensitivity analysis can be a time-consuming and tedious process. We suggest here a novel method for rapidly determining how changes in model parameters affect outputs. The technique involves randomizing a set of parameters, running repeated simulations, collecting results, then performing partial least squares regression using the randomized parameters and simulation results as input and output matrices, respectively. This process generates a simplified, empirical model that can predict the results obtained with a new set of input parameters. The regression coefficients in the simplified model indicate how changes in the parameters affect the model outputs. The utility of this method is demonstrated by randomizing maximal conductances and ion transport rates in several models of the ventricular action potential. The empirical linear models generated using regression are quite accurate despite the significant nonlinearities in the mechanistic models. The regression coefficients that indicate output sensitivities to parameters are surprisingly robust, even when parameters are varied over a wide range. Most important, the effects on model outputs identified through the regression procedure can identify counterintuitive aspects of model behavior. This new method therefore shows promise as a tool for the rapid characterization of computational models. This general strategy may also suggest methods for integrating traditional quantitative models with lower quality but large scale date sets obtained using new high-throughput technologies.

3006-Pos Ca Transient Alternans: The Roles of Sarcoplasmic Reticulum Ca Release, Uptake and Leak

Lai-Hua Xie, Daisuke Sato, Zhilin Qu, James N. Weiss

David Geffen School of Medicine at UCLA, Los Angeles, CA, USA.

Board B309

Beat-to-beat alternation of the amplitude of the cardiac Ca transient (CaT-Alt) plays a key role in the development of action potential duration alternans (APD-Alt). APD-Alt can create a highly arrhythmogenic substrate, especially when it becomes spatially discordant, promoting conditions favoring reentry and fibrillation. Therefore, understanding the mechanisms causing CaT-Alt, and its interaction with other factors contributing to APD-Alt, may have clinical implications for developing antiarrhythmic strategies. In the present study, we applied an iterative map approach to analyze the roles of the Ca-induced Ca release (CICR) gain from sarcoplasmic reticulum (SR), kinetics of SR Ca uptake, and SR Ca leak in the onset of CaT-Alt. We then compared the predictions by the iterative map to numerical simulations using a recently developed action potential (AP) model, as well as to AP clamp experiments in isolated rabbit ventricular myocytes. Both iterative map predictions and simulations agreed with the myocyte results, in which the pacing cycle length at the onset of CaT-Alt (226 ± 10 under control conditions) was:

(i) prolonged to 408 ±15 ms by increasing CICR gain with Bay K 8644 (100 nM);
(ii) decreased to 156 ± 6 ms by enhancing SR Ca uptake with overexpression of an adenoviral SERCA2a construct;
(iii) decreased to <150 ms by increasing SR Ca leak with FK506 (20 μM).

Although the iterative map predicted that increased SR Ca leak should enhance CaT-Alt if SR Ca content was maintained, this did not occur in either the model or experiment due to unloading of the SR Ca when leak was increased. Thus, the iterative map accurately predicts the dynamics controlling the onset of CaT-Alt in a realistic cardiac AP model and rabbit ventricular myocytes under control conditions and various interventions altering Ca cycling.

3007-Pos Angiotensin II Type 1 Receptor Partially Mediates Hypoosmotic-induced Increase of Guinea Pig Atrial IKs Current

Dimitar P. Zankov, Futoshi Toyoda, Mariko Omatsu-Kanbe, Hiroshi Matsuura, Minoru Horie

Shiga University of Medical Science, Otsu, Japan.

Board B310

Cardiomyocytes could repeatedly experience abnormal mechanical impact in the course of common heart disorders (systemic hypertension, ischemic heart disease), which produce stretch or swelling of myocytes. This could alter myocyte electrophysiology and are known to be arrhythmogenic. Slow component of I_K (I_{Ks}) - a major repolarizing K^+ current - is stimulated by stretch or swelling of cardiomyocytes through by far incompletely understood mechanism. Stretch was also found to activate AT_1 receptor in the absence of angiotensin II.

We studied involvement of AT_1 receptor in swelling-induced I_{Ks} increase in guinea pig atrial myocytes by the use of whole-cell patch-clamp method. Hypotonic solution (HS) with 0.7 osmolality enhanced atrial I_{Ks} 84.1±8.4% and contracted atrial action potential at 90% repolarization (APD_{90}) by 16.8±1.3% (n = 9). Selective block of AT_1 receptor with 1 or 5 μmol/L candesartan, or 5 μmol/L olmesartan attenuated HS-induced I_{Ks} increase to 48.0±4.1% (n = 10), 47.2±2.7% (n = 9), and 59.4±5.5% (n = 9), respectively. In addition, olmesartan reduced effect of HS on APD_{90} to 12.4±1.4% (n = 10, $P < 0.05$). Inhibitors of protein tyrosine kinases (TKs) tyrphostin A23 and A25 but not inactive analog A1 (all at 20 μmol/L) reduced I_{Ks} enhancement to 42.0±9.5 (n = 13) and 52.7±4.7% (n = 12), respectively. Inhibition of tyrosine phosphatases by 500 μmol/L orthovanadate promoted I_{Ks} stimulation by 0.7 HS to 124.6±10.6% (n = 8) and decreased recovery rate of tail current amplitude after HS withdrawal. Pharmacological suppression of G proteins, phospholipase C and PKC did not affect I_{Ks} modulation.

The above results implicate AT_1 receptor and TKs in the swelling-induced I_{Ks} activation in guinea pig atrium and suggest possible acute antiarrhythmic properties of AT_1 receptor blockers in the settings of stretch-related atrial arrhythmogenesis.

3008-Pos Intracellular Calcium Cycling Affects APD Restitution In Isolated Ventricular Myocytes But Not In Purkinje Cells Of The Mouse Heart

Elena G. Tolkacheva, Sandeep V. Pandit, Jose Jalife, Justus M.B. Anumonwo

SUNY Upstate Medical University, Syracuse, NY, USA.

Board B311

Introduction

Mouse models are important in the study of cardiac ion channel diseases and their electrophysiological consequences. However, little is known about the rate-dependence (restitution) of action potential duration (APD) of normal mouse cardiac cells. In addition, the restitution properties of isolated murine Purkinje cells are unknown. We investigated the effect of intracellular calcium ($[Ca^{2+}]_i$) cycling on APD restitution in both isolated murine myocytes and Purkinje cells.

Methods

Whole-cell current-clamping was used to record action potentials from paced cells. The pipette solution contained either 10 mM or 1 mM EGTA to achieve differing degrees of $[Ca^{2+}]_i$ chelation. APDs were measured at 90% repolarization (APD_{90}) at basic cycle lengths (BCL) varying between 1000 and 100 ms.

Results

In the presence of 10 mM EGTA, the APDs of both ventricular myocytes and Purkinje cells remain unchanged as the BCL is varied from 1000 ms to 100 ms. Purkinje cell APDs are significantly longer than ventricular myocytes APDs at all BCLs. In contrast, in the presence of 1 mM EGTA, the restitution properties of myocytes are different from those of Purkinje cells. The APDs of Purkinje cells remain unchanged, but the APDs of ventricular myocytes increase as the BCL is varied from 1000 ms to 100 ms. As a consequence, APDs of Purkinje cells are significantly longer than those of myocytes only at large values of BCLs (> 200 ms).

Conclusion

Our results indicate that intracellular Ca^{2+} cycling does not affect the restitution properties of Purkinje cells but significantly alters them in ventricular murine myocytes. The difference between APDs of murine myocytes and Purkinje cells is diminished under physiological conditions, i.e. high pacing frequency and in the presence of $[Ca^{2+}]_i$ cycling.

3009-Pos Cytosolic Ca^{2+} Buffering Modifies Intra-SR Ca Dynamics In Intact Beating Murine Hearts

Dmytro Kornyeyev, Yimei Du, Ariel Escobar

Texas Tech University Health Sciences Center, Lubbock, TX, USA.

Board B312

The regulation of Ca-induced Ca^{2+} release in cardiomyocytes strongly depends on the magnitude and the dynamics of myoplasmic $[Ca^{2+}]$ changes during the cardiac cycle. In this study we investigated the dynamic characteristics of Ca^{2+} release under conditions of Ca^{2+} buffering with a high affinity and low binding rate Ca^{2+} chelator (EGTA). The mouse hearts were loaded with rhod-2 AM to follow the cytosolic Ca^{2+} signal. Mag-fluo-4 AM was used as an effective indicator for the luminal Ca^{2+} signal. Ca^{2+} transients were recorded from the epicardial layer at 21 and 37°C. The data obtained imply that EGTA-treated hearts had shorter refractory period and accelerated restitution of Ca^{2+} release/depletion in both cytosol and lumen. For instance, the presence of 71 μM EGTA-AM in loading solution shortened the time for 50% peak recovery from 140±12 to 93±18 ms at the temperature of 37°C. However, the treatment with EGTA had opposite effects on the relaxation kinetics in two cell compartments mentioned above causing strong decrease in the time constant for fast component of the relaxation of rhod-2 signal. Restitution of action potential measured with floating intracellular microelectrode and Ca^{2+} influx recorded by blocking Ca^{2+} release channels with ryanodine during action potential were considerably faster than the restitution of Ca^{2+}-induced Ca^{2+} release process. Additionally, we used trains of stimulation pulses to partially deplete the Ca^{2+} stores in EGTA-treated hearts to certain level. A test stimulation pulse applied after different time periods after the train revealed that restitution of Ca^{2+} release depends on the diastotic Ca^{2+} in lumen, not on the time between stimulations. In addition, the presence of EGTA shifted appearance of the alternans towards higher stimulation frequencies.

Supported by NIH R01-HL-084487 and Cardiovascular SEED grant 7741-78-0420 to AE.

3010-Pos Ranolazine Modulates Action Potential Parameters Of Human Atrial Myocytes

Laura Sartiani[1], Silvia Suffredini[1], Simona Brogioni[1], Elisabetta Cerbai[1], Luiz Belardinelli[2], Alessandro Mugelli[1]

[1] *CIMMBA, University of Florence, Florence, Italy,*
[2] *CV Therapeutics, Palo Alto, CA, USA.*

Board B313

Increased late sodium current (INaL) may play a role in action potential (AP) prolongation and arrhythmogenesis. By inhibiting INaL, ranolazine (RAN) attenuates AP prolongation and reduces sodium/calcium overload in myocytes with enhanced INaL. No data exist about RAN effect in human cardiomyocytes. Electrophysiological effects of RAN (3–10μM) was assessed in human atrial myocytes (HuAM), from biopsies of patients undergoing cardiac surgery. APs stimulated at 0.2–1Hz were recorded in the perforated-patch configuration; duration was measured at −50mV (APD50) or 90% repolarization (APD90). Values are reported in Table as mean-SEM (*P<0.05, RAN vs. Control, n=13–18).

Effect of Ranolazine on human atrial myocyte action potentials

	Control (ms)	RAN 3μM (ms)	RAN 10μM (ms)
APD50 (0.2Hz)	227±51	173±28	121± 20*
APD50 (1Hz)	205±36	201±33	133±18*
APD90 (0.2Hz)	336±35	331±35	266±24*
APD90 (1Hz)	298±33	329±45	293±27

RAN shortened atrial APD of HuAM stimulated at 0.2Hz, whereas at 1Hz RAN prolonged APD90 at 3μM, but had no effect at 10μM. To test RAN effect in the setting of enhanced INaL, cells were exposed to anemone toxin (ATX-II, 10nM) and APs measured in the disrupted-patch configuration. ATX-II prolonged APD50 and APD90 by 469% and 282% at 0.2Hz, and by 332% and 236% at 1Hz, respectively (n=9, p<0.05). The effect was reversed by 10μM RAN (p<0.05). Thus, RAN shortens APD of HuAM by inhibiting INaL; this effect may result in an effective reduction in intracellular calcium overload.

3011-Pos Transmural Myocardial Gradients in Endogenous Angiotensin II regulate the Na/K Pump and Transient Outward K^+-Current

Junyuan Gao, Xiurong Sun, Jeremy Kim, Ira S. Cohen, Richard T. Mathias

SUNY at Stony Brook, Stony Brook, NY, USA.

Board B314

We previously reported a gradient in endogenous angiotesin II (A2) across the canine ventricular wall, with 1670 nM in endocardial (Endo) and zero in epicardial (Epi) myocytes. These concentrations were based on the transmural gradient in A2-mediated inhibition of Na/K pump current. We also reported that the transient outward K^+-current (I_{TO}) was higher in Epi than Endo, and I_{TO} in Endo could be increased to the value in Epi by inhibiting A2 receptors with losartan. In the present study, we investigated the transmural gradient in I_{TO} to predict endogenous A2. The canine ventricular wall was dissected into 5 regions: Epi, Mid-Epi, Mid-Center, Mid-Endo and Endo. I_{TO} was measured with the whole cell patch clamp and normalized to membrane capacitance. All values are given Epi to Endo. I_{TO} (pA/pF, mean±SD) was: control myocytes 10.9±1.1, 7.3±1.0, 5.4±0.9, 4.8±0.5 and 4.3±0.5; following inhibition by exogenous A2 (5 uM) 4.7±1.1, 4.6±0.6, 4.3±1.0, 4.3±0.4, and 4.1±0.7. Given the SDs, these values suggest uniform expression of I_{TO} when A2 is uniform. The dose-inhibition curve of I_{TO} by A2 was constructed in Epi cells, and yielded a best fit $K_{1/2}$ of 169 nM, and inhibition at 5 uM A2 of 56%. Endogenous A2-mediated inhibition of I_{TO} in the 5 regions was calculated from 56% minus the % inhibition by 5 uM A2; it was 0%, 31%, 46%, 51% and 54%. Based on these values, the transmural gradient in endogenous A2 is (nM): 0, 185, 583, 1033, and 1705. These concentrations are essentially the same as those based on gradients in Na/K pump current. The same transmural gradient in endogenous A2 appears to regulate both I_P and I_{TO}.

This work was supported by HL67101, HL28958 and HL85221.

3012-Pos Crosstalk Between IP3 And cAMP May Mediate The Actions Of Alpha1-Adrenoceptor Agonists In Guinea-pig Atrial Myocytes

Thomas P. Collins, Derek A. Terrar

Oxford University, Oxford, United Kingdom.

Board B315

IP_3 has been shown to increase the L-type Ca^{2+} current (I_{CaL}) in atrial myocytes (Wang *et al.* 2005). Recently, Ca^{2+} stimulated isoforms of AC have been identified in guinea-pig sino-atrial node cells where they make an important contribution to pacemaking (Mattick *et al.* 2007). These ACs were also identified in atrial myocytes. The aim of the present study was to determine whether IP_3 dependent Ca^{2+} release can regulate I_{CaL} through the actions of Ca^{2+}-stimulated ACs and protein kinase A.

Immunocytochemistry revealed that type 2 IP_3 receptors were located sub-sarcolemmally while Ca^{2+} stimulated ACs were associated with the sarcolemma. Chelation of intracellular Ca^{2+} with BAPTA-AM reduced peak I_{CaL} by 39±7 % (P<0.05, n=5) and increased the time to peak by 29±7 % (P<0.05, n=5). Superfusion with the AC inhibitor MDL had a very similar effect to BAPTA-AM as it reduced peak I_{CaL} by 40±5 % (P<0.01, n=6) and increased the time to peak by 39±10 % (P<0.05, n=6). As well as reducing I_{CaL}, MDL decreased Ca^{2+} transient amplitude and increased both the rise

time and the decay time. It thus appears that there is significant AC activity in atrial myocytes.

Phenylephrine increased atrial myocyte Ca^{2+} transients by 35±9 % ($P<0.01$, n=8). This effect was prevented by pre-treatment with prazosin (n=6), an α_1-adrenoceptor antagonist. The effects of phenylephrine were also prevented by 2-APB (n=5), a cell-permeant inhibitor of IP_3Rs, suggesting that IP_3 mediates the response to phenylephrine. In the presence of MDL, phenylephrine produced a 12±4 % ($P<0.05$, n=5) increase in Ca^{2+} transient amplitude. This increase was smaller than phenylephrine alone ($P<0.05$).

These observations are consistent with the actions of α_1-adrenoceptor agonists being mediated by IP_3-induced Ca^{2+} mobilisation and activation of Ca^{2+}-stimulated ACs.

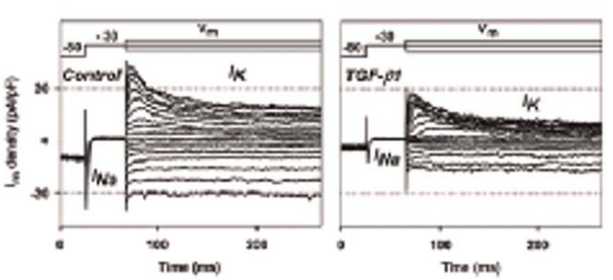
3013-Pos TGF-β1 Chronically Regulates Voltage-gated Sodium And Potassium Channels In Neonatal Rat Atrial Myocytes

Roberto Ramos-Mondragón, Esperanza Jiménez, Guillermo Avila

Cinvestav-IPN, Mexico DF., Mexico.

Board B316

TGF-β1 provokes cardiac structural remodeling. In contrast, little is known regarding electrophysiological effects. In this study, we have tested the hypothesis that TGF-β1 regulates sodium and potassium channels. Thus, we cultured atrial myocytes under either control conditions or the presence of 200 pM TGF-β1 (1–2 days). Subsequently, myocytes were subjected to whole-cell patch-clamp experiments, and sodium and potassium currents were investigated. TGF-β1 produced a significant decrease in the densities of inward ($I_{K\text{-}in}$; 55%) and outward sustained ($I_{K\text{-}sus}$; 50%) potassium currents, without significantly altering the transitory outward component (IK-to). TGF-β1 also decreased the density of I_{Na} available at −80 mV (80%), the corresponding maximal conductance (G_{max}; 50%), and charge movement (Q_{on}; 50%), without significantly affecting voltage dependence of activation. Analysis of sodium channels voltage-dependence of inactivation suggests that TGF-β1 reduces the number of channels recruited at very negative potentials (I_{max}; 30%), shifts inactivation curves by −7 mV, and does not affect the slope factor. Thus, TGF-β1 downregulates both sodium channel density in plasma membrane, and the fraction of noninactivated channels at −80 mV. These results support the notion that TGF-β1 has the potential to provoke cardiac electrical remodeling.



3014-Pos Optical Mapping Reveals Gradients of Action Potential Duration during Coronary Artery Occlusion in Isolated Rabbit Hearts

Soontaree Petchdee, Francis L. Burton, Godfrey L. Smith

Biomedical and Life Sciences, Glasgow, United Kingdom.

Board B317

Acute myocardial ischemia can induce ventricular arrhythmias, such as ventricular tachycardia and fibrillation. To study the electrical changes induced by acute local ischaemia, isolated Langendorff-perfused rabbit hearts were stained with the voltage sensitive dye RH237. Optical action potentials were recorded from a 16x16mm area of epicardium using a 16x16pixel photodiode array. The heart was immobilised with either 3- butane-dione monoxime (BDM, 15 mM) or blebbistatin (5 μM). A superficial coronary artery was occluded with a snare; within 3–4 mins of occlusion electrophysiological effects are evident in a region of the myocardium distal to the occlusion. Progressive decreases in action potential duration and upstroke velocity occurred over the subsequent 5–10 mins. These effects were reversed when the coronary artery occlusion was released. When BDM was used to reduce movement, a 10 minute period of ligation caused a decrease in action potential duration (at 50% repolarisation, APD_{50}) from 180±11ms (n=5) to 93±10ms. On recovery, APD_{50} returned to normal (167±7ms). Furthermore, the rise time of the action potential (time from 10% to 90% depolarisation) increased (from 5.4±0.3ms to 14±2ms). In the non-infarcted area of myocardium, no significant changes in APD_{50} or rise time were observed during the occlusion process. Similar results were observed using blebbistatin. In only one out of eight experiments, using 10 mins of occlusion, did arrhythmias occur during the occlusion. In contrast, global ischaemia invariably caused arrhythmias within 8–10mins. In conclusion, acute coronary artery occlusion produces dramatic gradients of action potential duration, but this alone is insufficient to cause arrhythmias.

3015-Pos Effects of Voluntary Exercise on Monophasic Action Potentials in Rats

Rachel E. Stones[1], Rudolf Billeter[2], Mark Drinkhill[1], Simon Harrison[1], Ed White[1]

[1] *The University of Leeds, Leeds, United Kingdom,*
[2] *The University of Nottingham, Nottingham, United Kingdom.*

Board B318

Exercise training can lead to electrical remodeling in mammalian hearts, including humans. We are investigating these changes using a female rat model involving 5–6 weeks of voluntary wheel running exercise which increases action potential duration (APD; Natali et al, 2002, J.Physiol. 541, 863–875) and a decrease in transient outward current (Ito) in single sub-epicardial (EPI) left ventricular myocytes from trained hearts. Isolated hearts were mounted on a Langendorff apparatus, perfused at 37°C and stimulated at a frequency of 5 Hz. Monophasic action potentials (MAPs) were recorded from the left ventricular epicardial surface. MAPs from

trained rats were significantly longer at 50, 75 and 90% repolarisation than those from sedentary rats. (*P<0.05, unpaired t-test, trained n=8, sedentary n=9). In addition we assessed the relative levels of expression of the genes encoding for Ito in the rat (Kv4.2, Kv1.4 and Kv4.3) and factors (KChIP2 and Irx5) which are implicated in the gradient of Ito across the left ventricular wall. Levels of mRNA for Kv4.2, KChIP2 and Irx5 were dependent upon region (EPI vs ENDO) but were not affected by voluntary exercise. We conclude there is electrical remodelling in the female rat heart in response to voluntary exercise training and that the prolongation of the EPI APD seen in single myocytes is not dispersed by electrotonic loading by adjacent cells in the whole heart. A decrease in EPI Ito current may explain the effect of voluntary exercise on APD. Our measurements of mRNA do not support a change in gene expression of the factors responsible for Ito, suggesting the mechanism of action may be a decrease in the number of functional channels in the membrane.

Funded by the British Heart Foundation and the Wellcome Trust.

3016-Pos Phenotype Of Transgenic Mice With Deletion Of Both Cardiac Calsequestrin And Triadin-1

Hyun Seok Hwang[1], Paul D. Allen[2], Bjorn C. Knollmann[1]

[1] *Vanderbilt University Medical Center, Nashville, TN, USA,*
[2] *Brigham and Women's Hospital, Boston, MA, USA.*

Board B319

Cardiac calsequestrin (Casq2) and its binding protein triadin-1 (Trdn) regulate ryanodine receptors (RyRs) and SR Ca^{2+} release in cardiac muscle. We previously reported that *Casq2* null (*Casq2*$^{-/-}$) mice display normal contractile function and morphology but exhibit cardiac arrhythmias after catecholamine challenge (= catecholaminergic polymorphic ventricular tachycardia, CPVT). Interestingly, triadin protein is significantly reduced in *Casq2*$^{-/-}$ mice, raising the possibility that reduction of triadin contributes to the CPVT phenotype. To test this hypothesis, we generated transgenic mice null for both Casq2 and Trdn (*Casq2*$^{-/-}$*/Trdn*$^{-/-}$). *Casq2*$^{-/-}$ (n=10) and *Casq2*$^{-/-}$*/Trdn*$^{-/-}$ (n=10) mice, 3–4 months of age, were compared with biometric, surface EKG, and echocardiography. The heart weight-to-body weight ratio was similar in both groups (*Casq2*$^{-/-}$: 0.51±0.06 mg/g vs. *Casq2*$^{-/-}$*/Trdn*$^{-/-}$: 0.53±0.06 mg/g, p=0.5). Heart rate, cardiac dimensions and fractional shortening were not significantly different between the two groups. *Casq2*$^{-/-}$ *Trdn*$^{-/-}$ mice exhibited a 7-fold higher rate of premature ventricular complexes (PVC) than *Casq2*$^{-/-}$ mice (7.1±7.1 PVCs/min vs1.1±1.4 PVCs/min, n=10 each, p<0.05 by Mann-Whitney-U Test) during maximal heart rate response during a 10 min period after injection of isoproterenol (1.5 mg/kg) under anesthesia. Ventricular tachycardia (VT) occurred in 2 of 10 *Casq2*$^{-/-}$ mice and in 6 of 10 *Casq2*$^{-/-}$ *Trdn*$^{-/-}$ mice (p=0.08). Although both *Casq2* $^{-/-}$ and *Casq2*$^{-/-}$ *Trdn*$^{-/-}$ mice have similar cardiac morphology and function, our data suggest that loss of Trdn exacerbates the CPTV phenotype of *Casq2*$^{-/-}$ mice. Thus, *Trdn* should be considered as a candidate gene in patients with the CPVT syndrome.

Supported by HL71670 and HL88635

3017-Pos Sex Differences in I_{CaL} in Adult Rabbit Right Ventricular Myocytes Modulate Gender Vulnerability to Early Afterdepolarizations

Carl Sims[1], Prakash C. Viswanathan[2], Daron J. Colflesh[3], Guy Salama[2]

[1] *Youngstown State University, Youngstown, OH, USA,*
[2] *University of Pittsburgh, Pittsburgh, PA, USA,*
[3] *Carnegie Mellon University, Pittsburgh, PA, USA.*

Board B320

Adult females have a longer QTc interval and greater susceptibility to arrhythmia in drug-induced Long QT syndrome type 2 (LQT2). Recent studies provide accumulating evidence that suggest factors other than APD and gonadal hormones modulate arrhythmia sensitivity. The L-type calcium current (I_{CaL}) is a key determinant of intracellular calcium homeostasis and has been implicated as a trigger of early afterdepolarizations (EADs) and Torsade de Pointes (TdP). We previously demonstrated that sex differences in the levels and biophysical properties of I_{CaL} from rabbit left ventricular myocytes was an underlying cause of EAD vulnerability in a rabbit model of LQT2. Therefore, using the patch clamp technique and cardiac AP modeling, we investigated whether gender differences in I_{CaL} levels in adult rabbit right ventricle myocytes might also underlie the sex gap in arrhythmia vulnerability. We found that $I_{Ca\text{-}L}$ density measured at 0 mV was 30.6% higher in female (11.28 ± 0.94 pA/pF, n = 8) compared to male myocytes (7.83 ± 1.23 pA/pF, n = 8, $p < 0.05$). No significant gender differences in $I_{Ca\text{-}L}$ activation or inactivation were observed, suggesting that sex differences exist only in current density and not channel properties. To determine the electrophysiological consequence of experimentally observed differences in current density between the male and female right ventricle, we incorporated the values into the Luo-Rudy action potential model. EADs were observed in the simulated female right ventricular myocyte, but not the male right ventricular myocyte following suppression of the rapid component of the delayed rectifier (I_{Kr}) by 75%. The higher $I_{Ca\text{-}L}$ density in female right ventricular myocytes may account for their enhanced vulnerability to arrhythmia due to $I_{Ca\text{-}L}$ reactivation or enhanced calcium overload which increases the propensity to EADs and TdP.

3018-Pos Ionic Currents in Isolated Mouse Cardiac Purkinje Cells

Ravi Vaidyanathan, Ryan O'Connell, Elena Tolkacheva, JustusM.B

Anumonwo. SUNY Upstate Medical University, Syracuse, NY, USA.

Board B321

Introduction: Purkinje fibers are vital for the conduction of the cardiac impulse in the ventricular myocardium; however, the ionic bases of the action potential in cells isolated from the Purkinje fibers (PC) are unknown.

Methods: PC and myocytes were obtained by enzymatic digestion (Langendorff) and comparatively studied using conventional patch-clamp techniques.

Results: PC were morphologically different from apical and septal cells and averaged (*um*) 129±7 in length and 8±0.3 in width (n=28). Whereas, apical and septal cells averaged, respectively, 104±2 and 110±2 in length and 27±0.9 and 25±0.7 in width (n=79,76). Action potential duration at 90% repolarization in PC (58±4 ms; n=10) was longer than in apical (32±4 ms; n=7) and septal (37±1 ms, n=4) myocytes. Spontaneous electrical activity was present only in PC consistent with the existence of the hyperpolarization-activated current (I_f). The inward rectifier current density was similar PC, apical and septal cells. In the presence of TTX and nisoldipine, 4.5 sec depolarization activated currents with different densities and kinetics in the three cell types. Peak current density at + 40 mV averaged (pA/pF) 18.7±2.7, 32.4±2.6, 25.7±2.5 in PC (n=6); apical (n=7); and septal (n=6) cells. Currents in apical cells and PC could be fitted with two exponentials (~50, ~1300 ms), whereas an additional exponential (~400 ms) was needed to fit currents in septal cells (see Xu et al, *JGP* 1999, 115). In addition to the L-type Ca^{2+} current, PC expressed the T-type Ca^{2+} current.

Summary and Conclusions: This is the first characterization of ionic currents in the mouse Purkinje cell. Our data show significant differences in ionic current mechanisms in PC and myocytes, which should be important in understanding impulse propagation in the mouse model.

3019-Pos Ischemic Conditions cause a Differential Depression of Sodium Current in the Canine Left Ventricle

Danielle M. Renodin, Lisa Murphy, Charles Antzelevitch, Jose M. Di Diego, Jonathan M. Cordeiro

Masonic Medical Research Laboratory, Utica, NY, USA.

Board B322

Background: Cardiac ischemia produces a greater depression in the upstroke velocity of the action potential in ventricular epicardium (Epi) compared to the endocardium (Endo). This basis for the differential effect on excitability was studied by recording the fast Na^+ current (I_{Na}) in Epi and Endo ventricular cells.

Methods: Canine Epi and Endo myocytes were isolated from the left ventricle. Whole cell voltage clamp methods were used to record I_{Na} in both cell types. I_{Na} was recorded in low external Na^+ to ensure adequate voltage control.

Results: Action potential recordings from the left ventricular wedge exposed to global ischemia showed a greater reduction in Vmax in Epi tissue. Patch clamp analysis of I_{Na} showed that from a holding potential of −120 mV, I_{Na} density was similar Epi and Endo cells (70.0±9.3 pA/pF versus 68.3±6.3 pA/pF respectively at −35 mV). Steady state inactivation of I_{Na} was more negative in Epi compared to Endo cells ($V^1/_2$=−83.6±0.1 mV for Epi and −75.5±0.3 mV for Endo, $p<0.05$). Recovery of I_{Na} was similar in the 2 cell types. Lowering extracellular pH (one component of ischemia) from 7.4 to 6.6 significantly reduced I_{Na} magnitude by 22.7% in Epi cells and 23.1% in Endo cells, but exerted no effect on steady state inactivation of I_{Na} or recovery from inactivation.

Conclusions: Our results indicate that there is a significant difference in I_{Na} channel availability between Epi and Endo cells, but no difference in current density or recovery from inactivation. External pH of 6.6 reduces sodium current in both Epi and Endo cells without affecting channel availability or recovery. These electrophysiological distinctions likely to contribute to the effect of acidosis and extracellular K^+ accumulation, two components of ischemia to preferentially depress excitability in ventricular epicardium.

3020-Pos Splenocytes Reprogrammed To A Pluripotent State By Fusion With ESCs Can Give Rise To Functional Cardiomyocytes In Vitro

Alexey Kuzmenkin[1], Yijun Yuan[1], Tobias Cantz[2], Martin Breitbach[3], Bernd Fleischmann[3], Hans Schoeler[2], Juergen Hescheler[1]

[1] *Institute of Neurophysiology, University of Cologne, Cologne, Germany,*
[2] *Max Planck Institute for Molecular Biomedicine, Muenster, Germany,*
[3] *Institute of Physiology I, University of Bonn, Bonn, Germany.*

Board B323

Due to a low regenerative potential of the adult mammalian heart, cells suitable for regenerative therapy of myocardial infarction are urgently needed. Embryonic stem cells (ESCs) can be differentiated into cardiomyocytes (CMs) and then used for transplantation, however, the applicability of this method is very limited due to the high risk of graft rejection, as well as due to justified ethical objections about consumptive use of embryos. Therefore, we applied a different approach based on reprogramming of terminally differentiated somatic cells (splenocytes) to a pluripotent undifferentiated state by fusion with ESCs and a successive generation of CMs suitable for cardiomyoplasty and displaying no immunological rejection. Intriguingly, it was unclear whether pluripotent clones generated by fusion can give origin to a functional cardiac tissue, and whether the generated tissue has the same physiological characteristics as the normal one. In our experiments, splenocytes were fused with HM-1 embryonic stem cells. After reprogramming to a pluripotent state, generated tetraploid fusion clones underwent cardiac differentiation when cultured as spheroids. Using whole-cell patch-clamp technique, we demonstrated that these clones can give rise to functional cardiomyocytes in vitro. We determined expression patterns, functionality, and sensitivity to blockers of voltage-gated Na^+, K^+, Ca^{2+} and HCN channels on the early and late stages of the differentiation process and compare them to ESC-derived as well as to fetal cardiomyocytes. Additionally, cardiomyocytes derived from fusion clones were tested for unimpaired humoral regulation (effects of isoproterenol and carbachol) and mature action potentials. Our project explores the applicability of cell fusion for generation of patient-specific cardiomyocytes and is of a high importance for regenerative medicine of heart disorders.

3021-Pos Mitochondrial Na^+/Ca^{2+} Exchanger Inhibition Improves NADH/NAD^+ Redox Balance In Failing Cardiomyocytes

Ting Liu, Brian O'Rourke

The Johns Hopkins University, Baltimore, MD, USA.

Board B324

The etiology of heart failure may include a mismatch in energy supply and demand. We previously demonstrated that elevated intracellular Na^+ impairs mitochondrial NADH production in paced cardiomyocytes by inhibiting mitochondrial Ca^{2+} accumulation. Treatment with a mitochondrial Na^+/Ca^{2+} exchange inhibitor, CGP-37157 reverses this defect. Here, we investigate

(i) whether Na^+_i is elevated in myocytes from failing guinea-pig hearts,
(ii) whether the NADH/NAD^+ balance is impaired during rapid stimulation, and
(iii) whether CGP-37157 can prevent the redox imbalance in heart failure.

Pressure overload heart failure was induced by ascending aortic constriction and cardiac function was monitored with echocardiography every 2 weeks. When a decrease in ejection fraction (EF) was observed (after ~6–8 weeks), animals were euthanized, heart weight/tibia length was measured, and cardiomyocytes were isolated. Compared to age-matched non-surgical controls, heart weight/tibia length of banded animals was increased by ~85% (from 0.50±0.04 to 0.93±0.16) while EF was decreased by 20% (from 62% to 50%). $[Na^+]_i$, measured ratiometrically using SBFI, was increased in failing cells (Control: 5.22±1.36mM; Failing: 16.81±3.13 mM). Myocytes were field stimulated at 4Hz (2mM $[Ca^{2+}]_o$; 100nM isoproterenol; 37°C) for 100 seconds and then returned to rest while NADH (expressed as % reduction) was continuously monitored and calibrated at the end of each experiment by exposure to FCCP (5μM; 0%) and NaCN (4 mM; 100%). In normal cardiomyocytes, NADH levels were maintained during 4Hz stimulation (pre-stimulation: 51±3%; end stimulation: 54±3%); however, in cardiomyocytes from failing hearts, NADH levels decreased dramatically during stimulation (pre-stimulation: 69±4%; end stimulation: 34±5%). Treatment of failing cells with CGP-37157 (10μM) prevented the decrease in NADH during rapid pacing (pre-stimulation: 71±2%; end stimulation: 72±4%). The findings support the idea that mitochondrial Na^+/Ca^{2+} exchanger could be a therapeutic target to improve the function of failing heart.

3022-Pos A Potential Mechanism By Which Embryonic Stems Cells Altering Native Cardiomyocytes Electrophysiological Properties

Priyanka Karan[1], Zhe Jiao[2], Shariar Irvanian[2], Hong Liu[2], Jon Allen[2], Jon Allen[2], Samuel Dudley[2]

[1] *Geogia Institute of Technology, Atlanta, GA, USA,*
[2] *Emory University, Atlanta, GA, USA.*

Board B325

Background - Embryonic Stem Cells (ESCs)are being considered for cardiac regeneration. We studied their electrophysiological implications using co-cultures of ESCs and neonatal rat ventricular myocytes (NRVM).

Methods and Results- Comparing cultures without and with 5% ESCs at 4 days, the mean bipolar field potential duration (FPD) of NRVMs increased from 26.3 ± 2.2 ms (n=10) to 44.3 ± 6.2 ms (n=9; $p < 0.05$), interspike interval (ISI) increased from 358.3 ± 62.8 ms (n=10) to 947.8 ± 214.6 ms (n=7; $p < 0.01$), and conduction velocity (CV) decreased from 14.2 ± 1.3 cm/s (n=8) to 4.6 ± 1.2 cm/s (n=5; $p < 0.01$). To evaluate ESCs paracrine effects on NRVMs, media conditioned by 3 million ESCs for 24 h was diluted 1:1 with fresh media (ESC CM). Conditioned media was changed daily and altered mean FPD, ISI, and CV to 46.1 ± 7.8 ms, ISI to 682.0 ± 128.5 ms, and 4.2 ± 0.4 cm/s (n=8; $p < 0.01$ for each measure), respectively at 4 days as compared to media treated similarly but with exposure to ESCs. Suggesting a potential mechanism for CV slowing, Western blots revealed an increase in dephosphorylated Cx43/unphosphorylated Cx43 ratio by 96% in the 5% mouse ESCs and 53% ESC CM as compared to controls (n=8; $p < 0.01$ for each). Cardiac sodium channel expression was a significant decreased to 64.9 ± 6.0% (n=8) and 73.8 ± 13.8 % (n=7) of the control for ESCs and ESC CM respectively. This correlated with a 38% reduction in INa.

Conclusions -ESCs decreased ISI, prolonged FPD, and slowed CV of NRVMs. This effect was mediated by a soluble factor. The mechanism for the CV increase may be related to reductions in Cx and Na+ channel activities.

3023-Pos The Use Of A hERG Frozen Cell Line On Different Automated Electrophysiology Platforms

Gary Clark[1], Scott Maidment[1], Corina Ehnert[2], Christa Nutzhorn[2], Andrew Southan[1]

[1] *BioFocus DPI, Saffron Walden, United Kingdom,*
[2] *Cytocentrics AG, Rostock, Germany.*

Board B326

Safety pharmacology profiling is an increasingly important step in the drug discovery process and the recent emergence of automated electrophysiology platforms has significantly reduced the resources required for hERG screening compared to the more traditional conventional electrophysiology evaluation. However; this approach requires optimisation of the cell line for good performance using a planar substrate, consistent current expression levels within the population of cells and significant amounts of tissue culture resource to maintain cells in passage. We have evaluated the use of a cryopreserved HEK293 hERG cell line optimised for planar substrate electrophysiology by Cytocentrics AG. Such cryopreserved cells should limit variability in expression levels often observed using growing cultures, significantly reduce tissue culture resource requirements and enable experiments to be scheduled at short notice. The cell line was initially validated using conventional electrophysiology and the DynaFlow system (n= 26 cells); the cryopreserved cells expressed mean peak tail current of 1.0nA and

gave IC50 values for quinidine and cisapride of 284nM and 12nM, respectively. Using IonWorks Quattro recording in single hole mode, 78% of cells were expressed tail currents greater than 0.15nA with mean peak tail current being 0.35nA (n=3 PatchPlates). This compares to typical values of 37% and 0.49nA (n=3 PatchPlates) for a HEK293 hERG cell line maintained in growing culture. We will present data to show that planar substrate recordings from the cryopreserved hERG cell line give appropriate pharmacological potency values and perform in a more consistent manner than HEK293 hERG cells maintained in growing culture.

3024-Pos Role of S6 residues in hERG gating and pharmacology

Sarah Dalibalta, Rachael H. Hardman, John S. Mitcheson

University of Leicester, Leicester, United Kingdom.

Board B327

hERG (*human ether-a-go-go related gene*) encodes a voltage-gated K^+ channel characterised by slow activation and deactivation gating, but rapid and voltage dependent inactivation. Pharmacological block of hERG is associated with arrhythmias and sudden death. Alanine scanning mutagenesis studies of the pore inner helices (S6) identified V659A as a mutant that dramatically slows deactivation and also reduces the potency of hERG blockers. Val659 is located close to where the S6 helices bundle together in the closed channel. In the current study, Val659 was substituted for different amino acids and the effects on gating and pharmacology investigated.

The V659A mutation increases the slow time constant of hERG deactivation from the *wild-type* (WT) value of 349 ± 30 ms to 3181 ± 156 ms. It has previously been shown that the amino- (N−) terminus of hERG slows deactivation by interacting with the pore to stabilise the open state. N-truncation (NTK) of V659A hERG resulted in faster deactivation kinetics (*tau* slow = 701.54 ms) than V659A, but deactivation kinetics were not as fast as NTK-hERG; thus Val659 may form part of the receptor for N-terminus binding in the open state. Replacing Val659 with large hydrophobic residues (Phe, Ile, Trp) resulted in deactivation kinetics similar to *WT* hERG indicating the channel can close relatively normally and suggesting this position is orientated away from the bundle crossing in the closed state. Interestingly, V659G currents did not deactivate at potentials as negative as −180 mV, suggesting an uncoupling between the voltage sensor and channel pore. Inhibition of Val659 mutants by ibutilide was loosely correlated with deactivation. We conclude that Val659 mutations impact on deactivation through interactions with the voltage sensing domain (perhaps the S4-S5 linker) and have only allosteric effects on drug binding.

3025-Pos Functional Regulation of Cardiac KCNQ1 Potassium Channel by Association of KCNE3

Futoshi Toyoda, Dimitar P. Zankov, Wei-Guang Ding, Hiroshi Matsuura

Shiga University of Medical Science, Otsu, Japan.

Board B328

Cardiac KCNQ1 potassium channel assembles with KCNE1 to generate the slow I_{Ks} current that plays a major role in repolarizing the cardiac action potential. It is a plausible hypothesis that other KCNE members (i.e. KCNE2 through KCNE5) may interact with cardiac KCNQ1 to form non-I_{Ks} potassium currents because they are also expressed in heart. In the present study, regulation of cardiac KCNQ1 channel by KCNE1 and KCNE3 was investigated using a patch-clamp technique. Transfection of KCNE1 into CHO cells stably expressing KCNQ1 resulted in evocation of slowly activating outward current resembling cardiac I_{Ks}, whereas expression of KCNE3 induced constitutively active non-I_{Ks} potassium current. When both KCNE1 and KCNE3 were cotransfected, an ensemble of KCNQ1/KCNE1 and KCNQ1/KCNE3 currents was revealed, suggesting that KCNE3 can participate in regulation of KCNQ1 channel even in the presence of KCNE1. To further clarify the roles of KCNE1 and KCNE3 for cardiac membrane currents and action potentials, expression of each KCNE gene in guinea-pig ventricular myocytes was suppressed by RNA interference. In cells transfected with small interfering RNA directed against KCNE1, I_{Ks} amplitude was largely reduced by approximately 70% and action potential duration (APD) was significantly increased. On the other hand, knockdown of KCNE3 resulted in prolongation of APD without changing I_{Ks} amplitude. Taken together these results suggest that not only KCNE1 but also KCNE3 may play a physiological role in repolarization of cardiac action potentials.

3026-Pos Effects of a KCNA5 Mutation in a Human Atrial Myocyte: A Computational Study

Gunnar Seemann[1], Daniel L. Weiss[1], Eberhard P. Scholz[2], Frank B. Sachse[3], Olaf Dössel[1]

[1] *Institute of Biomedical Engineering, Universität Karlsruhe (TH), Karlsruhe, Germany,*

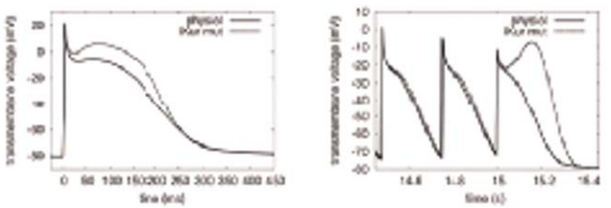
[2] *Dept. of Cardiology, Medical University Hospital Heidelberg, Heidelberg, Germany,*

[3] *Nora Eccles Harrison Cardiovascular Research and Training Institute, Univ. of Utah, Salt Lake City, UT, USA.*

Board B329

Atrial fibrillation (AF) is a frequently occurring disease associated with increased risk of stroke and ventricular arrhythmia. A recent study identified a familial type of AF caused by a loss-of-function mutation of the ion channel underlying the cardiac IKur current [1]. The critical effect of this mutation is the generation of early afterdepolarizations that raise the risk of AF. We studied the effects of this mutation on action potentials (AP) in a computational model of human atrial electrophysiology. The conductivity of IKur was reduced in the Courtemanche et al. model [2] to the heterozygously measured values of 38%. The AP duration of the mutant cell was prolonged from 198 to 215ms and the AP had a more pronounced plateau (left figure). With increased stimulus frequency, the computational cell started early afterdepolarizations above 4Hz (right figure). This effect is driven by calcium induced depolarization

effect. The simulation results demonstrated a complementary way how a loss-of-function mutation can influence electrophysiology of cardiomyocytes and how this might induce AF.



References

[1]. Olson TM et al. Hum Mol Genet. 2006; *15*(14):2185–2191
[2]. Courtema nche M et al. Am J Physiol. 1998; *275*(44):H301–H321

Smooth & Skeletal Electrophysiology

3027-Pos Familial Hypokalemic Periodic Paralysis - The Catastrophe on the Cusp of Weakness

Michael Fauler, Frank Lehmann-Horn, Karin Jurkat-Rott

Ulm University, Ulm, Germany.

Board B330

Familial Hypokalemic Periodic Paralysis is an inherited muscle disease. Patients suffer from episodically occurring attacks of flaccid muscle paralysis, lasting hours to days. These episodes go along with an shift of potassium ions into muscle. Attacks are triggered by stimuli, that increase cellular uptake of potassium, e. g. insulin, adrenaline and rest after exercise. To relieve paralysis therapeutically, a large, overshooting administration of potassium, to increase its extracellular concentration, is needed. This does not result in a gradual increase of force, but a sudden jump from weak to strong, indicative of hysteresis. It is well known, that the paralysis is due to membrane inexcitability, caused by a pathologic depolarization. This has long been puzzling, since the known genetic defects in the genes, coding either for the voltage-gated sodium ($Na_V1.4$) or the voltage-gated calcium channel ($Ca_V1.1$), cause a loss-of-function in the channel proteins. Recently, evidence has been presented, that mutant channels are leaky for sodium, potassium or protons (Sokolov et al, 2007, Struyk et al, 2007). By using methods of systems dynamics, we analysed the effects of such a leak current on the behaviour of a mathematical model of an excitable cell. Bifurcation analysis reveals a cusp catastrophe as the manifold, that describes the dependency of equilibrium states on the extracellular potassium concentration and the size of the leak current. This elementary catastrophe can explain main features of the disease: bistability (strong vs. weak), hysteresis (potassium administration leads to a sudden increase of strength) and the occurrence of permanent weakness with no prior episode of weakness. Unclarities remain on the existence and meaning of compensatory mechanisms, the location of the resting state on the manifold, the quantitative impact of hysteresis and the exact mode of jumping between states.

3028-Pos Voltage-gated Ca^{2+} Currents In Smooth Muscle Cells Of Arterioles From Leg Muscles Of Mouse

Alexandra Uliyanova[1], Roman Shirokov[2]

[1] *UMDNJ GSBS, Newark, NJ, USA,*
[2] *UMDNJ NJMS, Newark, NJ, USA.*

Board B331

Voltage-dependent Ca^{2+} currents are important for the myogenic tone and contractile responses of the smooth muscle cells (SMCs) in the resistance arteries of skeletal muscle. However, they have not been measured directly due to the difficulty of isolation of these SMCs. We succeeded to record voltage-dependent Ca^{2+} currents in single SMC of arterioles (20–40 μm diameter) from semitendinosus and biceps femoris muscles. The SMCs were not separated from the arteriole. Instead the electrical coupling between cells was prevented by various techniques.

With 20 mM Ca^{2+} in the bath, maximal currents (+30 mV) were 12±7 pA/pF (n=4). When 10 μM of nifedipine was added to the solution, currents peaked at +10 mV and their maximal density decreased to 2.3±0.3 pA/pF (n=3). The nifedipine resistant current could be further blocked by 45±13 % (n=3) by addition of 40 μM of Ni^{2+}. The magnitude of the low-voltage Ni^{2+}-sensitive component changed little when Ba^{2+} substituted extracellular Ca^{2+}. Based on these properties, this current is likely to be through the T-type Ca^{2+} channels. Although the presence of dihydropyridine-resistant Ca^{2+} channels in SMCs of resistance arterioles of skeletal muscle had been proposed previously, this is the first report of their significant contribution to Ca^{2+} currents in these cells.

3029-Pos AAV Delivery of the BK Channel Gene to Vascular Smooth Muscle as a Long-lasting Antihypertensive Strategy

Sung W. Rhee, Keshari Thakali, Russell B. Melchert, Li Pang, Terry W. Fletcher, Kerrey A. Roberto, Paul L. Hermonat, Nancy J. Rusch

University of Arkansas for Medical Sciences, Little Rock, AR, USA.

Board B332

Essential hypertension is a polygenetic disease afflicting nearly 1 billion individuals worldwide. Of the individuals offered antihypertensive drugs, only one third achieve blood pressure control due to the high cost, side effects and lack of adherence to the daily, multi-drug therapy that is often required. In this regard, the long-term expression of vasodilator proteins would be extremely advantageous to avoid the high cost and lack of adherence to daily drug regimens, and to minimize blood pressure fluctuations caused by short-acting antihypertensive drugs. Of major interest as a candidate vasodilator protein is the high-conductance, Ca^{2+}-activated K^+ (BK) channel that is expressed in the surface membrane of vascular smooth muscle cells (VSMCs). The BK channel is activated by rises in intravascular pressure, and mediates compensatory vasodi-