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(1mM) was used to rapidly and reversibly block the RyR. The tetracaine-dependent shift of Ca2+ from the cytosol to the SR is proportional to diastolic Ca2+ release. Primary myocytes were treated with 250nM ISO with and without Akt inhibitor-X (AIX; 5μM; 30min pre-incubation and continuous perfusion). ISO-induced increase in the diastolic SR Ca2+ release was abolished by the treatment of myocytes with AIX. When data were selected such that SR[Ca2+] was matched in each group (ISO: 155.22 ± 5.1μM; ISO+AIX: 153.92 ± 3.8μM), myocytes treated with ISO had significantly higher tetracaine-dependent increase in diastolic SR[Ca2+] (12.43 ± 3.8μM) vs. those treated with ISO and AIX (1 ± 2.2μM) (P=0.01, t-test). The results suggest that the ISO-dependent increase in diastolic SR Ca2+ release is dependent upon Akt. To further test this hypothesis myocytes were cultured for 24 hours with adenovirus to express a dominant-negative Akt construct (Akt-dn). Preliminary evidence shows reduced diastolic SR[Ca2+] release for a given SR[Ca2+] in Akt-dn myocytes. This evidence indicates that Akt activation may be an important upstream effector involved in the β-AR-induced diastolic SR Ca2+ release.

2600-Pos Board B370

Ranolazine Alters the Properties of Localized Ca^{2+} Sparks and Inhibits Global Ca^{2+} Waves in Rat Myocytes

Derek Steele, Zhaokang Yang.
University of Leeds, Leeds, United Kingdom.

The effect of ranolazine (10 μM) on local Ca^{2+} regulation was studied in isolated rat ventricular myocytes. Cells were loaded with fluo-4 AM and Ca^{2+} was detected using line-scan confocal imaging. Ranolazine increased Ca^{2+} spark frequency by 57.8 ± 17% ($p<0.05$, n=29). This was accompanied by significant ($p<0.05$) decreases in Ca^{2+} spark amplitude (44.5 ± 0.1%), duration (16.5 ± 1.9%) and width (5.6 ± 1.7%) resulting in a decrease in the calculated 'spark mass' of 41.3 ± 5.4% (data from 15-29 cells). In cells initially paced at 0.2 Hz in the presence of forskolin (10 μM), cessation of stimulation was followed by spontaneous diastolic Ca^{2+} waves. After introduction of ranolazine (but not in its absence), the frequency of spontaneous Ca^{2+} waves decreased markedly over 10-15 minutes and waves were completely abolished in 5 of 11 cells. Inhibition of Ca^{2+} waves also occurred in permeabilized cells, suggesting an intracellular site of action. We propose that the ranolazine-induced decrease in Ca^{2+} spark mass may contribute to inhibition of pro-arrhythmic Ca^{2+} waves by reducing the probability of saltatory propagation between Ca^{2+} release sites.

2601-Pos Board B371

Voltage Dependence of Calcium Spike Characteristics in Rat Cardiac Myocytes

Matej Hotka, Radoslav Janíček, Alexandra Zahradníková, Jr., Eva Poláková, Alexandra Zahradníková, Ivan Zahradník.
Institute of Molecular Physiology and Genetics, SAS, Bratislava, Slovakia.

Integral calcium release fluxes in mammalian cardiac myocytes increase in amplitude not only due to the increased recruitment of individual release sites but also due to their increased synchronization (1). Here we characterize the voltage dependence of synchronization of individual release sites at high temporal resolution. Calcium spikes and calcium currents elicited in isolated rat ventricular myocytes (2) by 80 ms voltage pulses from −50 to −40 - +50 mV were recorded using Leica TCS AOBS confocal microscope (2 kHz line scan mode) and Axopatch 200B patch-clamp amplifier, respectively. Calcium spikes were identified and analyzed by a new Matlab-based software that highly increased the productivity and reliability of approximation of calcium spikes by the kinetic model of local calcium release (2). Calcium spikes were described by their amplitude, onset latency, time-to-peak, FDHM, time constants of activation and termination, and fractional probability of activation. The synchrony of calcium spikes increased with depolarization and could be fully explained by the voltage dependence of their onset latency that reached a lower limit of about 4 ms at depolarizations between 0 and 40 mV. The rate of calcium spike activation after its onset was independent of the applied voltage or of calcium current amplitude, while the probability of calcium spike activation was related to the voltage dependence of the integral of calcium current. We conclude that both the synchrony of local calcium release activation and recruitment of individual release sites are fully controlled by the activation probability of DHPR calcium channels and by the DHPR-RYR coupling fidelity.

Supported by APVV-0721-10, VEGA 2/0203/11, VEGA 2/0917/11.

1. Song LS, Sham JS, Stern MD, Lakatta EG, Cheng H. J Physiol. 512:677-91, 1998.
2. Zahradníková A Jr, Poláková E, Zahradník I, Zahradníková A. J Physiol. 578:677-91, 2007.

2602-Pos Board B372

Refractoriness of Calcium Release Units in Rat Cardiac Myocytes

Radoslav Janicek, Alexandra Zahradníková, Jr., Eva Poláková, Alexandra Zahradníková, Ivan Zahradník.
Institute of Molecular Physiology and Genetics SAS, Bratislava, Slovakia.

Calcium release units (CRUs) in cardiac myocytes contain a large number of ryanodine receptors (RyRs), but it seems that only a small fraction of RyRs is being activated during a single calcium release event. Repetitive activity of CRUs is not well understood from the point of refractoriness. We used direct measurement of Ca-spikes activated by calcium current (ICa) to unveil the refractoriness of CRU firing from the properties and occurrence of calcium spikes under control conditions and in the presence of the DHPR activator FPL.
ICa was activated by voltage pulses from 50 to 0 mV under whole-cell patch-clamp. Ca-spikes were measured by confocal microscopy using Ca2+ indicators (Fluo-3 and Oregon Green BAPTA-5N) in the presence of EGTA. A large majority of CRUs responded to stimulation by a single Ca-spike, while 13.4 % produced two subsequent (twin) Ca-spikes. Early Ca-spikes (single spikes and the first of twin Ca-spikes) had similar latencies, while the second Ca-spikes were significantly delayed. Amplitude distribution of the early Ca-spikes consisted of four quantal levels with equal amplitudes and binomially distributed frequency of occurrence, supporting the hypothesis that different Ca-spike amplitudes are due to a different number of independent quantal levels, likely individual RYR openings. The probability of occurrence of second Ca-spikes was inversely proportional to the quantal size of the early Ca-spike. In the presence of FPL, the probability of occurrence of twin spikes was decreased relative to control, despite persistent activation of DHPR receptors.
We conclude that refractoriness of local calcium release does not result from Ca-dependent DHPR inactivation, but rather it is caused by inactivation of the release unit due to local depletion of the SR.

Supported by APVV-0721-10, APVV-0441-09, VEGA 2/0203/11, VEGA 2/0917/11, and VEGA 2/0190/10.

2603-Pos Board B373

Cyclopiazonic Acid Differently Modulates Junctional and Non-Junctional Ca^{2+} Transients in Rat Atrial Myocytes

Joon-Chul Kim, Nana Kang, Pyung Keun Myung, Sun-Hee Woo.
Chungnam National University, Daejeon, Korea, Republic of.

Atrial myocytes have two functionally distinguished sarcoplasmic reticulums (SRs): those at the periphery close to the cell membrane, and those at the cell center (interior) not associated with the cell membrane. To know the extent to which the peripheral and central Ca^{2+} release sites are controlled by SR Ca^{2+} pump the effects of cyclopiazonic acid (CPA), the inhibitor of SR Ca^{2+} pump, on peripheral junctional Ca^{2+} releases and central non-junctional Ca^{2+} releases were compared. Rapid (230 Hz) 2-D confocal Ca^{2+} imaging was used in field-stimulated rat atrial myocytes. Diastolic Ca^{2+} level was increased by CPA dose-dependently. At lower doses (0.2 and 0.4 μM), the CPA-induced increase in diastolic $[Ca^{2+}]$ was larger in the center. Low dose of CPA increased the systolic Ca^{2+} level and magnitude of Ca^{2+} transients shortly in the cell periphery. However, high concentration (5 μM) of CPA significantly decreased them in the cell periphery. In contrast, low doses of CPA increased central Ca^{2+} transients slowly and the effect was prolonged. High dose of CPA did not reduce central Ca^{2+} transients. Ca^{2+} transient decay was slowed by CPA dose-dependently. The CPA-induced deceleration of Ca^{2+} transient decay was significantly stronger in the center than in the periphery. The velocity of Ca^{2+} propagation wave during action potential was dose-dependently reduced by CPA. This result was consistent with more increase in the time-to-peak of central Ca^{2+} release. Our data suggest that the Ca^{2+} pump may play more important role in non-junctional Ca^{2+} decay in beating atrial myocytes. In addition, our data suggest that active Ca^{2+} uptake via the SR Ca^{2+} pump may facilitate the transverse Ca^{2+} propagation wave in atrial myocytes on depolarization.

2604-Pos Board B374

Eccentric Exercise Elicits Elevation of Resting Calcium in Skeletal Muscle. Periodic Acceleration Enhanced the Recovery

Jose R. Lopez[1], Jose Mosquera[2], Alfredo Mijares[3], P.D. Allen[1].
[1]Brigham and Women's Hospital, Boston, MA, USA, [2]CIM, Mexico, Mexico, [3]CBB, IVIC, Caracas, Venezuela, Bolivarian Republic of.

Eccentric exercise (EC) can cause transient muscle damage, including muscle injury, muscle protein release into the plasma, an acute-phase immune response, and a decrease of muscle performance. Periodic acceleration (pGz) is a novel treatment that stimulates nitric oxide (NO) production by cNOS. Male mice (25-32 g) were divided in 4 groups (5/group). *Group A*: non-exercised (control); *Group B*: EC (45 min downhill exercise); *Group C*: EC followed by 30 min of pGz at 480 cpm daily starting on day 0 for 10 days; *Group D*: EC followed by 30 min of pGz, treated with L-NAME, 4 days prior and

10 days after EC. Resting intracellular Ca^{2+} concentration ($[Ca^{2+}]_{rest}$) was measured *in vivo* using Ca^{2+} selective microelectrodes on days 0, 2, 4, 6, 8, and 10 after EC. In ***group A*** the average $[Ca^{2+}]_{rest}$ was 1113.8 nM on day 0 and did not change on subsequent days. On days 0, 2, 4, 6, 8 and 10 $[Ca^{2+}]_{rest}$ (in nM) in polarized-exercised muscle fibers in ***group B*** was 395 ± 38, 381 ± 35, 334 ± 23, 295 ± 39, 268 ± 27, and 223 ± 27. In ***group C*** (EC+pGz) $[Ca^{2+}]_{rest}$ was 402 ± 46, 304 ± 26, 260 ± 35, 145 ± 16, 121 ± 10 nM and 113 ± 6, showing a significant difference from group B by day 2 and reached control by day 10. In ***group D*** (EC+pGz+LNAME) the beneficial effect of pGz on $[Ca^{2+}]_{rest}$ was abolished. We conclude *that* EC induced a chronic elevation of $[Ca^{2+}]_{rest}$ in skeletal muscle that lasted up to 10 days and that pGz was able to accelerate the return to normal $[Ca^{2+}]$ homeostasis following EC induced muscle injury. The salutary effect of pGz on $[Ca^{2+}]_{rest}$ appears to be mediated by an increase in NO generation, since the NOS blocker, L-NAME, eliminated the effect of pGz on $[Ca^{2+}]_{rest}$ after EC.

2605-Pos Board B375

Basal PKC Activity Regulates Spontaneous Firing of Cardiac Pacemaker Cells

Tatiana M. Vinogradova, Edward G. Lakatta.
NIA, NIH, Baltimore, MD, USA.

Spontaneous beating of sinoatrial node cells (SANC) is linked to rhythmic, submembrane local Ca^{2+} releases (LCR) from the sarcoplasmic reticulum (SR). LCRs activate an inward Na^{+}/Ca^{2+} exchange current, imparting an exponential increase to the later diastolic depolarization, to bring membrane potential to the threshold and fire an action potential (AP). Previously we have demonstrated that basal PLC activity plays a key role in spontaneous firing of the cardiac pacemaker via regulation of SR Ca^{2+} cycling, but not IP_3 receptor Ca^{2+} release. The specific mechanisms that underlay PLC-dependent modulation of spontaneous SANC firing are still unclear. Here we show that SANC pacemaker activity is critically dependent on downstream target of PLC, i.e. protein kinase C (PKC). In freshly isolated rabbit SANC inhibition of PKC activity by GF109203X (10 μmol/L) markedly suppressed both spontaneous SANC beating by ~70% and LCR's parameters (confocal microscopy, Fluo-3 as Ca^{2+} indicator). Specifically, GF109203X decreased the LCR size (from 5.8 ± 0.3 to 2.8 ± 0.3 μm) and number per each spontaneous cycle (from 1.4 ± 0.2 to 0.7 ± 0.1); increased the LCR period, i.e. the time from the prior AP-induced Ca^{2+} transient to the subsequent LCR. The increase in LCR period during PKC inhibition predicted an increase in the spontaneous cycle length. All effects of GF109203X were reversed upon washout. Since Ca^{2+} cycling in SANC is critically dependent on L-type Ca^{2+} current ($I_{Ca,L}$), which contributes to the AP upstroke and modulates the SR Ca^{2+} content, we studied effects of GF109203X on $I_{Ca,L}$. PKC inhibition markedly suppressed $I_{Ca,L}$ amplitude (from 8.1 ± 1.0 to 4.6 ± 1.0 pA/pF), strongly suggesting that $I_{Ca,L}$ could be a major target of basal PKC activity in SANC. Thus, basal PLC-dependent regulation of spontaneous SANC firing is fulfilled through basal PKC-dependent modulation of Ca^{2+} cycling, specifically LCR's parameters and $I_{Ca,L}$ amplitude.

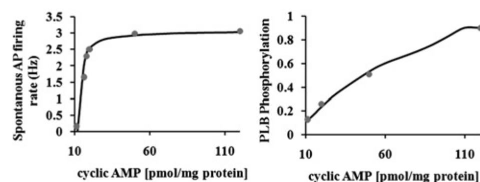
2606-Pos Board B376

How Ca^{2+}-Activated, cAMP/PKA-Dependent Phosphorylation Signaling Mediates Pacemaker Cell Activity: Experimental and *In Silico* Biochemical and Biophysics Perspectives

Yael Yaniv, Victor A. Maltsev, Edward G. Lakatta.
NIA/NIH, Baltimore, MD, USA.

Ca^{2+}-activated, adenylate cyclase (AC)-cAMP/PKA-dependent phosphorylation of both surface membrane electrogenic proteins of (''Membrane-clock''), and of intracellular proteins that generate rhythmic Ca^{2+} oscillations (''Ca^{2+}-clock''), regulate the periodicity of each clock, and couple the two-clocks to regulate sinoatrial node cells (SANCs) normal automaticity.

We developed a novel numerical model to simulate the coupling of SANC **Ca^{2+}-AC-cAMP/PKA** signaling to functions of surface membrane and Ca^{2+} cycling molecules. The model incorporates experimentally measured a term for Ca^{2+}-dependent AC-activity, and when Ca^{2+} changes predicts resulting changes in downstream cAMP/PKA phosphorylation-dependent signaling that produce changes in ion channels conductance and intracellular Ca^{2+} kinetics that ultimately change the spontaneous action potential (AP) firing rate.



Model predictions of Ca^{2+}-dependent changes in the cAMP/PKA phosphorylation cascade, of spontaneous AP firing rate and of the stoichiometry-relationships between cAMP, phospholamban phosphorylation and AP firing rate (line in the figure) faithfully reproduced the experimentally measured variables and their stoichiometry (points in the figure).

The simulations of this novel integrative model of biochemical and biophysical signaling within the coupled-clock model further support the importance of high throughput signaling via Ca^{2+}-AC-cAMP-PKA phosphorylation cascade in normal SANC automaticity.

2607-Pos Board B377

Crosstalk Between Calcium and cAMP in Pancreatic Alpha Cells

Nancy M. Lorenzon, Julie Imseis, Liang Liang, Colette Crane,
Larissa Ikenouye, Monika Pauckova, Joseph K. Angleson.
University of Denver, Denver, CO, USA.

Glucagon secretion from pancreatic alpha cells can be controlled by a variety of factors including hypoglycemia-induced increase in calcium or by hormones that stimulate cAMP production. The mechanisms by which calcium and cAMP signals influence each other and glucagon secretion have yet to be resolved. Using fura2 imaging, we found that acute hypoglycemia-triggered calcium-induced calcium release (CICR) in clonal mouse alpha cells was sensitive to the calcium channel blockers, nifedipine and w-conotoxin-GVIA. CICR with similar channel pharmacology was triggered by forskolin-induced elevation of [cAMP] revealing cAMP-stimulation of $[Ca^{2+}]_i$. This CICR likely involved multiple cAMP targets as both PKA-selective and EPAC-selective cAMP analogs each triggered CICR. In addition, a role for calcium regulation of [cAMP] was revealed by hypoglycemia-triggered increase in [cAMP] as reported by imaging of an EPAC-based cAMP FRET indicator. This was presumably due to calcium regulation of adenylyl cyclase or cAMP phosphodiesterase (PDE). Multiple isoforms of adenylyl cyclase and PDEs including Ca-sensitive AC3 and PDE1 were detected by RT-PCR in alpha cells. PDE3- and PDE4-selective inhibitors had distinct effects on calcium and cAMP dynamics and whole cell calcium currents. These data suggest multiple points of cAMP and calcium crosstalk in regulating glucagon secretion.

2608-Pos Board B378

Subcellular Modeling of PKA Activation and cAMP Diffusion in Localized Microdomains of Adult Cardiomyocytes

Britton W. Boras, Andrew McCulloch.
San Diego, San Diego, CA, USA.

The beta adrenergic pathway in cardiomyocytes activates protein kinase A (PKA) to phosphoregulate several Ca^{2+} handling proteins, including the L-type Ca channel, ryanodine receptor, and sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) (via phospholamban), resulting in inotropic, lusitropic and chronotropic responses. However, based on PKA kinetics measured *in vitro*, almost all PKA should be activated by basal concentrations of cAMP. Several recent studies have postulated a role for local degradation of cAMP by phosphodiesterases (PDE) in maintaining microdomains with lower cAMP concentrations. However, the degradation rate of cAMP is slow compared with the rate of cytosolic diffusion, suggesting that local clustering of PDE is insufficient to maintain the cAMP microdomains alone. In this study, Virtual Cell, a finite volume solver, was used to create 3D diffusional models that were then validated with *in vitro* FRET experiments to probe other potential mechanisms of PKA's compartmentalized response. We examined the effects of structural obstruction, cAMP buffering by exchange protein directly activated by cAMP (EPAC), PKA isoform localization, ion gradients, and physical coupling between PKA and PDE, mediated by A-kinase anchoring protein (AKAP). Our results suggest that structural obstructions, obtained from cryo-TEM images, are insufficient to decrease the rate of diffusion of cAMP. However, PKA isoform localization around the SERCA pumps is essential in maintaining phosphoregulation. Furthermore, AKAP complexes and basal ion concentrations also contribute to localized control of PKA. These *in vitro* and *in silico* experiments help us understand how microdomains in adult cardiomyocytes are maintained.

2609-Pos Board B379

EPAC Activation of Cardiac Muscle

Sarbjot Kaur, Cherrie H.T. Kong, Mark B. Cannell, Marie Louise Ward.
University of Auckland, Auckland, New Zealand.

Exchange protein-directly activated by cAMP (EPAC) has been shown to be involved in the classical cAMP-activated protein kinase A (PKA) pathway in many different cell types. However, in the cardiac muscle the effects of EPAC have yielded contradictory results with reports of increased (Oestrich, 2007) and decreased (Pereira, 2007 & Cazorla, 2009) Ca^{2+} transient amplitude in mice and rats, respectively. The aim of this study was to examine the role of EPAC activation in excitation-contraction (EC) coupling in isolated rat ventricular muscle preparations. To activate the EPAC pathway, we used