between basal state of LCR period (time from the prior AP-induced Ca^{2+} transient to the diastolic LCR) and AP cycle length (APCL) (interval between AP-induced Ca^{2+} transients) and its regulation by PKA-CAMKII dependent phosphorylation in single, isolated rabbit SANC. The role of LCRs in pacemaker activity of guinea-pig SANC or even their presence in the basal state has been recently challenged, but not systematically tested. To probe whether the guinea-pig SANC generate spontaneous diastolic LCRs and their role in the pacemaker firing, LCRs and beating rate in guinea-pig (n=8) were compared to that in rabbit SANC (n=43) at 35.5C.

Rhythmically beating guinea-pig SANC do, indeed, generate spontaneous diastolic LCRs beneath sarcolemma, detected via line-scan confocal imaging (fluo-4AM,10uM) when the scanline was positioned beneath sarcolemma parallel to the long axis of the cell. Furthermore, the average LCR characteristics and the and amplitude of the LCR Ensemble Ca^{2+} signal, the fundamental LCR parameter that ''talks to'' NCX during diastole were comparable in guinea-pig and rabbit SANC (Table). Relationship between LCR period and APCL were subtended by the same linear function (R2=0.95).

That LCRs in both guinea-pig and rabbit SANC contribute to the late diastolic depolarization and regulation of APCL indicate that coupled clock functions involved in pacemaker function are not species limited.

Table

	guinea-pig	rabbit
LCR amplitude (F/F0)	1.30 ± 0.04*	1.43 ± 0.03
LCR size(μm)	7.36 ± 1.20***	4.92 ± 0.15
LCR duration(ms)	39.78 ± 5.83	6.89 ± 1.51
LCR per cycle(Hz/100μm)	6.10 ± 1.44**	16.23 ± 1.51
LCRCa2+ signal(μmxmsxΔF/F0)	38.38 ± 9.90	28.30 ± 3.42
LCR Ensemble Ca^{2+} signal (product of μmxmsxΔF/F0)	320.24 ± 95.63	236.84 ± 37.51
LCR period(ms)	310.38 ± 41.88	315.09 ± 12.19
APCL(ms)	338.02 ± 34.09	378.92 ± 9.79

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New Targeted Ca^{2+} Probes Reveal Mitochondrial Ca^{2+} Signaling Plays a Critical Role in Rat Sinoatrial (SA) Nodal Pacing

Xiaohua Zhang[1], Christine Couch[1], Katalin Torok[2], Martin Morad[1].
[1]Cardiac signaling center of USC, MUSC, & Clemson University, Charleston, SC, USA, [2]Institute of Cardiovascular and Cell Science, St George's, University of London, London, United Kingdom.

It remains uncertain to what extent cardiac pacing is governed by ''membrane'' or ''Ca^{2+} clocks'' and what in fact is the master oscillator of the heart? As we have already reported that rapid release of mitochondrial Ca^{2+} is critical in spontaneous beating of neonatal rat cardiomyocytes, we investigated whether a similar mechanism is at play in SA-nodal cells. Adult rat SA-nodal cells were enzymatically isolated and were cultured for 12 hours prior to viral infection with two genetically encoded fluorescent mitochondria-targeted Ca^{2+} probes GCaMP3, Kd 1 & 3μM. After 2-3 days in culture, the targeted GCaMP3-probes produced the typical punctate mitochondrial pattern in spontaneously beating myocytes. Such cells had robust expression of If, ICa, and INCX. Simultaneously recorded TIRF images of cytosolic (Cai, dialyzed K5Fura-2) and mitochondrial (Cam, GCaMP3 probes) Ca^{2+} distributions in blebistatin-treated cells showed differential patterns of rise and fall of Calcium. It was noted that only ~50% of spontaneously beating SA-nodal cells expressed If when hyperpolarized negative to −70mV. Under current-clamp conditions, spontaneously triggered action potentials accompanied by in-phase Cai and Cam signals were recorded only in cells dialyzed with low concentrations of Ca^{2+} buffer (0.1 mM EGTA & Ca^{2+}). Withdrawal of extracellular Na^+ increased the rate of spontaneously generated Cai-oscillations at fixed holding potentials, but triggered diverse signals in different mitochondrial populations that ranged from continuous uptake of Ca^{2+} to spontaneously triggered oscillations to releasing of Ca^{2+}. Similarly, application of caffeine during Na^+ withdrawal while increasing Cai, showed Cam signals indicative of up-take of Ca^{2+} at some locations and release at others. We conclude that SR and mitochondrial Ca^{2+} signals and not If serve as the master oscillator of SA nodal cells. Supported by NIH Ro1HL-15162

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Preveleged Ca^{2+} Signaling Pathway between Membrane NCX and Mitochondria in Cardiac Myocytes

Xiaohua Zhang, Naohiro Yamaguchi, Lars Cleemann, Martin Morad.
Cardiac Signaling Center of USC, MUSC, & Clemson University, Charleston, SC, USA.

Accumulating evidence suggests that mitochondrial Ca^{2+} signaling plays a critical role in cardiac myocytes E-C coupling and that this may involve Ca^{2+} entering the mitochondria via the mitochondrial Ca^{2+} uniporter (MCU) and exiting on the mitochondrial Na^+-Ca^{2+} exchanger (mNCX). Here we investigate whether there are privileged Ca^{2+} transport pathways between surface membrane, mitochondria and SR. To address this issue, we used genetically engineered mitochondrial Ca^{2+} probes based on GCamP3 and GCamP6 along with synthetic fluorescent dye, Fura-2, to image mitochondrial and cytosolic Ca^{2+} movements simultaneously. Mitochondrial Ca^{2+} probes were virally introduced in either adult or neonatal cardiomyocytes. Whole-cell patch clamping and high-resolution TIRF microscopy were used to measure membrane current, mitochondrial Ca^{2+} and subsarcolemmal cytosolic Ca^{2+} simultaneously. In whole cell clamped myocytes dialyzed with 2mM EGTA, Na^+ withdrawal- (substituting Na^+ by tetraethylammonium, TEA) generated an outward NCX-mediated current reflecting Ca^{2+} entry and caused a rapid increase of mitochondrial Ca^{2+}, without significant rise of cytosolic Cai, because of Ca^{2+}-buffering by 2mM EGTA. When Na^+ was readmitted, causing a rapid fall (release) of mitochondrial Ca^{2+} it triggered a significant transient rise of cytosolic Ca^{2+} despite the presence of buffering by 2mM EGTA. Interestingly, 5mM caffeine puffs while triggering rapid release of Ca^{2+} from the SR caused little or no significant rise in the mitochondrial Ca^{2+} signal. Blockers of MCU or mitochondrial NCX failed to suppress this Na^+ withdrawal-induced mitochondrial Ca^{2+} rise. Our results suggest that Ca^{2+} buffers may block transfer of Ca^{2+} from the SR via the cytosol to mitochondria without blocking the privileged Ca^{2+} communication between sarcolemma and mitochondria. Supported by NIH grant R0-1,HL15162

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Binding Sites of the Ca/Na Exchanger NCX Analyzed with Poisson Fermi Theory

Jinn-Liang Liu[1], **Robert S. Eisenberg**[2].
[1]National Hsinchu University of Education, Hsinchu, Taiwan, [2]Rush University, Chicago, IL, USA.

The calcium sodium transporter NCX is a Y shaped branched channel in the structure crystallized by Liao et al. (2012) with well-defined binding sites. It seems unlikely that mechanism can be understood until structure is extended by estimates of binding free energies. It seems that existing molecular dynamics cannot cope with trace concentrations and movements of calcium. The number density of ionizable side chains is extremely high and so theory must include finite size ions and large electric and steric free energies. We use Fermi-Poisson theory (Liu and Eisenberg, 2014) that deals successfully with bulk solutions and several channel types. Outputs of theory include electric and steric fields of ions with different sizes; correlations of ions of different charge, and polarization of water, along with number densities of ions, water molecules and interstitial voids. These vary with conditions. They are not assumed.

We calculate electrostatic and steric potentials of three Na^+ and one Ca^{2+} binding site of the Liao structure with charges from PDB2PQR software. Atomic and numerical details of binding sites illustrate the ion-exchange mechanism and its energetic endpoints. Energy profiles of Na^+ and Ca^{2+} ions along their pathways give insight into the mechanism of coupling by which NCX moves intracellular Ca^{2+} uphill against its chemical gradient, while Na^+ moves downhill. But the mechanism and energetics of the conformation change of the transporter protein NCX remain a fascinating mystery.

NCX does not use energy beyond ion gradients and electric and steric fields described consistently by Poisson-Fermi theory. Its conformation change is that of catalysis, not pumping, in the dictionary sense of 'pumping'. Poisson-Fermi can describe energetic end points, although a kinetic model of conformation remains outside its grasp.

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Neuronal Intracellular Ca^{2+} and Na^+ Dyshomeostasis in the MDX Mouse

Jose R. Lopez[1], Juan Kolster[2], Jose Adams[3].
[1]Molecular Biosciences, University of California, Davis, CA, USA, [2]Centro de Investigaciones Biomédicas, Mexico, Mexico, [3]Division of Neonatology, Mount Sinai, Medical Center, Miami, FL, USA.

Duchenne muscular dystrophy (DMD) is an inherited X-linked disorder characterized by the deficiency of dystrophin as well as intracellular ion (Ca^{2+} and Na^+) dyshomeostasis in skeletal and cardiac muscles. There is also an absence of dystrophin in cortical neurons of DMD patients and animal models. We hypothesized that similar to muscles, intracellular ion dyshomeostasis may also occur in cortical neurons of mdx mice. We measured resting intracellular $[Ca^{2+}]i$ and $[Na^+]i$ in primary cortical neurons from 3-month-old mdx mice and age-matched wild type (wt) using Ca^{2+}- and