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Numerical Modeling of Flash-Induced Ca^{2+} Release in Sa Node Cells (SANC) Supports the "Coupled-Clock" Hypothesis of Cardiac Pacemaker Cell Function

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Our recent experiments in SA node cells using flash-induced Ca^{2+} release from a caged buffer (NP-EGTA) demonstrated that intracellular Ca^{2+} regulates normal SANC automaticity on a beat-to-beat basis. It cannot be directly envisioned, however, to what extent the reduction in action potential (AP) cycle length upon acute Ca^{2+} photo-release is attributable to rapid augmentation of Ca^{2+} pumping into sarcoplasmic reticulum (SR) followed by local Ca^{2+} releases (LCRs) vs. a direct effect of the flash-released Ca^{2+} on sarcolemmal proteins. To gain mechanistic insight into the problem, we numerically modeled the effects of flash-released Ca^{2+} using the 2009-Maltsev-Lakatta model of rabbit SANC that portrays pacemaker cell function as ''coupled-clock'' system, i.e. coupled intracellular Ca^{2+} oscillator of the SR and a cell membrane voltage oscillator. Knowing (from prior studies) Ca^{2+} ''on'' and ''off'' rates of NP-EGTA and experimentally measured AP cycle length increase following incubation with the buffer, by varying [NP-EGTA]i in model simulations we estimated [NP-EGTA]i (~8 mM) required to reproduce experimental results. With this estimated [NP-EGTA]i, we further estimated the rate of Ca^{2+} release during flash photolysis (a function of laser intensity) to be 1 μM/ms in order to reproduce experimental results on acute AP cycle length shortening. The model also faithfully reproduced diastolic Ca^{2+} release increase (assessed experimentally as LCR signal mass) following the flash-photolysis. Other numerical models based on a dominance of the membrane voltage oscillator, including the most recent and advanced Kurata et al. model and ''Kyoto'' model (developed by the Noma's group), could not reproduce the experimental results with any reasonable [NP-EGTA]i (up to 10 mM) and flash-induced Ca^{2+} release flux (i.e. laser intensity). Our numerical model simulations support the ''coupled-clock'' hypothesis of cardiac pacemaker cell function.

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Contribution of Calcium Waves to the Regulation of Beating Rhythm in Sinoatrial Node Cells

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The synchronized release of calcium (Ca) from the sarcoplasmic reticulum (SR) can initiate a heart beat. This concordant behavior can be altered and produced by the oscillatory dynamics of intracellular Ca release events (Ca waves) that can modulate membrane depolarization and hence heart rate. In this work, the effect of Ca waves on interbeat intervals (IBIs) was evaluated by using a stochastic simulation of rabbit SA-node cell which integrated a Ca wave model (Maltsev et al., 2011) including 1,350 discrete Ca release units (CRUs) with a classical membrane voltage oscillator (M clock model: Wilders et al., 1993). Each CRU opening produces a release flux equal to 1.0 pA and has a fundamental refractory period (varied from 220 to 300 ms), meant to empirically mimic beta-adrenergic effects on CRU intrinsic firing.

In the absence of SR function, the intrinsic IBI (due to membrane currents) is 450 ms. This IBI is reduced when stochastic CRU openings are allowed. As refractory period is reduced from 300 to 220 ms IBI progressively shortens to 431 ms, but this effect is small compared to the abbreviation in intrinsic CRU refractory period. This reflects the dynamic interplay of the Ca clock and membrane current clock, and also the stochastic nature of concerted CRU recruitment among neighboring CRUs that are required to drive sufficient inward Na/Ca exchange current and accelerate the IBI. Thus Ca waves with high rhythmicity pushes the cell into a state that is more ''ready to fire''. Detailed parameters and formulations from both individual models will impact the relative impact of Ca waves on IBI.

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Induced Pacemaker Cells Created by In Vivo Somatic Reprogramming: Phenotypic Comparison with Native Sinoatrial Node Cells

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Background: We report elsewhere in this meeting (N. Kapoor et al. abstract) that Tbx18, an embryonic T-box transcription factor, reprograms cultured cardiomyocytes to induced sinoatrial node (SAN) pacemaker cells. Here we sought to test the fidelity of reprogramming by expressing Tbx18 in adult ventricle and comparing the properties of Tbx18-transduced ventricular myocytes (Tbx-VMs) with those of GFP-transduced ventricular myocytes (as controls; control-VMs) and native SAN cells.

Methods: Adenoviruses co-expressing Tbx18 and GFP, or GFP alone, were directly injected in the apex of the guinea pig heart (4×10^7cfu/heart). Five days later, the heart was harvested and cardiomyocytes were isolated from the site of virus injection.

Results: Native SAN cells are smaller and leaner than nontransduced VMs: length-to-width (LtW) ratios equal 14.7 ± 1.5 (n=28) and 7.6 ± 0.7 (n=9), respectively. Freshly-isolated control-VMs maintained their native shape (LtW=7.4 ± 0.9, n=4); in contrast, Tbx-VMs were leaner (LtW=16.0 ± 1.0, n=12) than control-VMs and were often spindle-shaped, reproducing the morphological hallmark of SAN cells. Control-VMs had stable resting potentials at −76mV, with action potentials (APs) elicited only upon electrical stimulation. In contrast, spindle-shaped Tbx-VMs demonstrated diastolic depolarization (maximal diastolic potential=-59mV) and fired spontaneous APs at 26bpm.The whole-cell capacitance (a measure of cell size) of Tbx-VMs was smaller than that of control-VMs (40.8 ± 3.6 vs. 119 ± 16pF, respectively), but similar to that of native SAN cells (48.3 ± 7.0pF). The electrophysiological and morphological features of Tbx-VMs generally resembled those of native SAN cells.

Conclusion: Somatic gene transfer of Tbx18 in the ventricle *in vivo* yielded induced SAN (iSAN) cells which faithfully recapitulate the key phenotypic properties of genuine SAN cells. The *in situ* reprogramming was effective and swift (as little as five days), offering a novel approach to creating a biological pacemaker as an alternative to electronic devices.

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Lineage Reprogramming from Cardiomyocytes to Pacemaker Cells via a Single Transcription Factor

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The heartbeat originates in a small number of highly-specialized pacemaker cells found in the sinoatrial node (SAN). In an effort to create pacemaker cells from working cardiomyocytes, we expressed Tbx18, a transcription factor indispensible for embryonic development of the SAN. Within days, Tbx18-transduced neonatal rat cardiomyocytes (NRVMs) exhibited rhythmic spontaneous electrical firing. The mechanism of induced pacemaker activity resembled that of SAN cells, with enhanced voltage- and Ca^{2+}-dependent ''clock'' pathways. The maximum diastolic potential was more positive in Tbx18-NRVMs in comparison to controls (-47 ± 10 mV vs. -73 ± 6 mV) with accompanying reduction in I_{K1}. Line-scan confocal imaging of Tbx18-NRVMs loaded with Rhod-2 resolved localized Ca^{2+} release events (LCRs) that preceded each whole-cell Ca^{2+} transient resembling LCRs observed in native SAN cells. LCRs were not detected in control NRVMs. Spontaneous Ca^{2+}-transients in Tbx18-NRVMs were suppressed on superfusion with ryanodine (10 μM) by 47 ± 6% in comparison to 12 ± 2% suppression in controls, indicative of increased relevance of $[Ca^{2+}]_i$ stores. A 2.3-fold increase in $[Ca^{2+}]_i$ stores was observed in Tbx18-NRVMs which can be attributed to increased sarcoplasmic reticulum Ca^{2+}-ATPase 2a (SERCA2a) activity due to downregulation or phosphorylation of phospholamban (PLN), an inhibitor of SERCA. Tbx18-overexpression led to relative increase in the protein level of phosphorylated PLN (Ser16) similar to higher p-PLN level observed in the adult SAN relative to the left ventricle. In addition, Tbx18-NRVMs exhibited increased levels of cAMP and HCN4, shrank in size with disorganized myofibrils, and underwent epigenetic modifications consistent with durable reprogramming. Focal Tbx18 gene transfer in the guinea-pig ventricle consistently created ectopic pacemaker activity *in vivo* with a rhythm of 40 ± 20 beats/min (n=5/7). Taken together, the data demonstrate faithful reprogramming of ventricular myocytes to pacemaker cells by a single transcription factor, Tbx18.

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Enhanced Embryonic Stem Cell Differentiation to Cardiac Pacemaker Cells by Transduction with a Single Transcription Factor

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Background: Embryonic stem cells (ESCs) can spontaneously differentiate into heterogeneous aggregates of cardiomyocytes with atrial, ventricular, and