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Yin and Yang of the cardiac pacemaker clock system in health and disease

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Cardiac arrhythmias in general, and atrial fibrillation (AF), in particular, are important global health problems. Despite extensive studies, arrhythmia mechanisms remain unclear. The problem is complicated because heart function is affected by a complex integration of numerous biochemical and biophysical processes within and among cardiac cells. Interactions within SA node, the primary hearts' pacemaker that initiates and regulates the cardiac rhythm, results in one such critical unsolved complexity. Joung et al. ¹ have recently applied a method of simultaneous recording of intracellular Ca^{2+} and membrane potential to approach the riddle of complex/intimate interactions between electrophysiology and intracellular Ca^{2+} signaling within cells comprising SA node. In this issue of *HeartRhythm*, Joung et al. ² combined their method with measurements of expression of Ca^{2+} cycling proteins to explore the mechanisms of SA node dysfunction in AF.

Voltage-gated sarcolemmal ion currents are the proximal cause of an action potential (AP) as originally described by Hodgkin and Huxley based on voltage clamp data. *In silico*, the ensemble, or system, of the sarcolemmal electrogenic molecules (ion channels and transporters) of cardiac pacemaker cell can generate rhythmic APs, e.g. in 12 SA node cell (SANC) numerical models ³. Therefore, this system of ion currents can be envisioned as a membrane voltage oscillator or “membrane clock” (M clock). The classical perspective on cardiac pacemaker cell function is that the M clock is the ultimate cardiac pacemaker clock, i.e. its function is not only necessary, but also sufficient to drive normal automaticity. However, in addition to an M clock, cardiac pacemaker cells have another intrinsic oscillatory subsystem that resides within the cell: the sarcoplasmic reticulum (SR) pumps and periodically releases Ca^{2+} ⁴. While a potential role of the Ca cycling in normal function of cardiac pacemaker cells had been envisioned ^{5,6} and experimentally demonstrated ⁷⁻¹⁰ long ago, recent extensive studies (during last decade) have discovered the spatiotemporal characteristics of the SR Ca^{2+} cycling and its interactions with the M clock (review ¹¹). Confocal measurements in isolated pacemaker cells detect Localized submembrane Ca^{2+} Releases (LCR's) generated by the SR via ryanodine receptors (RyRs) during the late diastolic depolarization (DD) ^{12,13}. The integrated Ca^{2+} signal of these multiple LCRs during DD represents diastolic Ca^{2+} release (Fig.3 in ¹⁴) that was observed by Joung et al. ¹ as Late Diastolic Ca^{2+} elevation (LDCAE) in the primary region (i.e. impulse initiating part) of the isolated intact dog SA node. The spontaneous Ca^{2+} releases are referred to as an intracellular “ Ca^{2+} clock” because their occurrence is periodic during voltage clamp ¹⁵, in detergent-permeabilized SANC ¹⁶, and in

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silico (when membrane currents set to zero)¹⁷. In nature, i.e., in spontaneously firing SANC, in contrast to *in silico* or skinned or voltage-clamped cells, Ca^{2+} and M clocks do not exist in isolation of each other: Numerous and complex interactions via membrane voltage, submembrane Ca^{2+} , and protein phosphorylation occur between the two subsystem clocks (M clock and Ca^{2+} clock), and the subsystems become mutually entrained forming the full pacemaker cell SYSTEM or the master pacemaker clock (reviews^{11,18}). The two interacting clock subsystems do not just simply coexist within the pacemaker system but their interaction confers robustness and flexibility to the cardiac pacemaker function as discussed in a recent review¹¹ and demonstrated in a numerical study that predicts LDCAE¹⁷. Specifically, the presence of Ca^{2+} clock extends the fail-safe variations of membrane clock parameters, such as L-type Ca^{2+} current (I_{CaL}) conductance and, vice versa, the presence of some membrane components, such as funny current (I_f), increases the fail-safe variations of Ca^{2+} clock parameters, such as Ca^{2+} pumping rate¹¹.

In the context of the concept of a coupled clock system, the finding of Joung et al study² that RyR2 are down regulated, and LDCAE are absent in their experimental AF model, can be interpreted to indicate that the Ca^{2+} clock subsystem is impaired in AF, resulting probably in decreased robustness and flexibility of the pacemaker system. In other words, the impairment of diastolic Ca^{2+} releases shifts the operation of SANC likely towards less safe (i.e. susceptible to arrhythmia) operation.

But what about SANC M clock changes in AF? A recent study by Yeh et al.¹⁹ has identified that major electrophysiological changes of SANC in experimentally induced AF in dogs include a 50% reduction in I_f conductance and a 33% reduction in slow delayed rectifier K^+ current (I_{Ks}). Their numerical simulations showed that I_{Ks} change had almost no effect on SANC rhythm (less than 1% cycle length change) and the I_f change resulted in a cycle length increase of ~9%. But a change of this magnitude seems to be a relatively moderate effect, i.e. far from trouble. However, taking into account that the robustness of coupled clock system had been compromised by Ca^{2+} clock impairment identified by Joung et al² and discussed above, is it possible that this I_f change could be critical for SANC function? We believe that the answer to this question is not trivial, and presently can be approached only by numerical integration of changes of both Ca^{2+} and M clocks. Accordingly, we used our recently developed prototype model of interacting Ca^{2+} clock and M clock in rabbit SANC¹⁷ and performed numerical simulations to illustrate that it is indeed possible, at least at the level of a single pacemaker cell (Fig.1). The 50% reduction in I_f conductance produced a moderate rate reduction of the simulated AP firing rate (i.e. similar to numerical modeling by Yeh et al.¹⁹). The absence of Ca^{2+} release, with I_f remaining intact, however, substantially slowed the rate by ~40%, but AP firing still remained rhythmic (Fig.1C). However when the “impaired” Ca^{2+} release was **combined** with the “impaired” I_f function, the spontaneous beating became irregular.

These simple “first order” estimates illustrate that:

1. interactions of subsystem clocks are crucial to the generation of spontaneous APs of a normal rate and rhythm, and
2. when both M clock and Ca clock are impaired in AF (or in any other experimental or pathological conditions), uncoupling of M clock and Ca clock can push the master pacemaker clock towards its limits of fail safe operation.

Specific, detailed mechanisms of the system changes in AF and their numerical integration, specifically in canine SANC and SA node (and, ultimately, in human SA node), however, merit further studies. These additional mechanisms include characterization and integration of components of PKA and CaMKII-dependent phosphorylation (e.g. phosphorylation of phospholamban, SERCA, RyR, L-type Ca^{2+} channels), sarcolemmal ion exchangers (e.g.

Na⁺/Ca²⁺ exchanger and Na⁺/K⁺ pump), ion channel kinetics, intracellular contacts (e.g. via connexins), and mechanical factor, i.e. strain. An additional important requirement is a further improvement of both selectivity and spatiotemporal resolution of LDCAE recording within SA node. Such improvements will permit a determination of whether LDCAE exists during basal beating in large animals like canines as the case for rabbit and mouse, as well as whether LDCAE can propagate within the SA node. An intriguing question is also how expression of sarcolemmal electrogenic molecules (ion channels and transporters) and Ca²⁺ cycling proteins (especially RyR as found by Joung et al. ²) is regulated, the nature and kinetics of the molecular dysregulation in AF, and whether, in particular, changes in RyRs and AF are concomitantly reversed by cessation of chronic pacing.

In summary, the study by Joung et al. ² in this issue of *Heart Rhythm* suggests that rhythm disturbances in canine experimental AF are caused, at least in part, by a failure of Ca²⁺ clock function, specifically its release from SR via RyR2. However, the extent to which this mechanism contributes to AF requires further studies aimed to integration of changes in Ca²⁺ cycling proteins, their phosphorylation status, and changes in sarcolemmal electrogenic molecules. We believe that an important lesson from the recent papers by Joung et al. ^{1,2} and Yeh et al. ¹⁹ and our simple simulations (Fig.1) is that to be informative, future studies of cardiac pacemaker function, either normal or abnormal, must include integration of the two mutually entrained subsystems, Ca²⁺ clock and membrane clock, the Yin and Yang of the cardiac pacemaker cell function.

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Figure 1.

The combined impairment of M clock and Ca^{2+} clock in AF can result in generation of irregular action potentials by SA node cells. Simulations by a recent numerical model of rabbit SANC¹⁷ illustrate spontaneous action potentials (top traces), funny current (I_f , middle traces), and Ca^{2+} release flux from junctional SR (bottom panels) in control (A), 50% reduction in I_f conductance (B), no Ca^{2+} release (C), and 50% reduction in I_f conductance combined with no Ca^{2+} release (D). The original model¹⁷ was slightly modified to increase basal I_f conductance to 0.23625 nS/pF. See text for details.