

EDITORIAL COMMENT

# Mind the Gap

## Does Junctophilin 2 Gear the Coupled-Clock System in Pacemaker Cardiomyocytes?\*

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The processes underlying the initiation of the heartbeat have always been a major focus of cardiac research. The most recent consensus view on cardiac automaticity postulates that spontaneous, stable, and regular firing of pacemaker cardiomyocytes is due to a gradual change (namely, diastolic depolarization) in the membrane potential ( $V_m$ ) mediated by a complex system (or a coupled-clock model) that synergistically integrates electrogenic membrane proteins (membrane clock component) with subcellular  $Ca^{2+}$  handling machinery ( $Ca^{2+}$  clock component). The spontaneous diastolic depolarization is initiated by a hyperpolarization-activated HCN current ( $I_f$ ) and a low-voltage-activated T-type  $Ca^{2+}$  current ( $I_{Ca,T}$ ). Then, spontaneous, local subsarcolemmal  $Ca^{2+}$  release (LCR) events occur from the sarcoplasmic reticulum (SR) via ryanodine receptors (RyR2), which leads to the activation of an inward  $Na^+/Ca^{2+}$  exchange (NCX) current ( $I_{NCX}$ ) contributing to the late phase of diastolic depolarization. This boost in diastolic depolarization reaches the activation threshold for L-type  $Ca^{2+}$  current ( $I_{Ca,L}$ ) that initiates the pacemaker action potential (AP). Although it has been suggested that stable pacemaking relies on the complex spatiotemporal synchronization between the components of

the coupled-clock system, the mechanisms behind this interaction remain elusive.

*Jph2*-encoded Jph2 (junctophilin 2) is a structural protein that plays a critical role in the excitation-contraction coupling and  $Ca^{2+}$  regulation within the contractile myocytes of the heart. Jph2 provides a structural bridge between the sarcolemma and SR in atrial and ventricular working myocytes, thereby supporting functional coupling between L-type  $Ca^{2+}$  channels and RyR2 at dyadic junctions for robust  $Ca^{2+}$ -induced  $Ca^{2+}$  release (CICR) and a subsequent contraction.<sup>1</sup> Jph2 has also been shown to directly bind to and inhibit RyR2 function, including spontaneous  $Ca^{2+}$  release event from the SR. Until recently, it was unknown whether Jph2 can contribute to cardiac automaticity and provide structural and/or function coupling between the sarcolemmal and SR membranes, as it does in the contractile myocytes.

In a recent study, Landstrom et al<sup>2</sup> developed a tamoxifen-inducible, pacemaker tissue-specific, knockdown mouse of Jph2 using a Cre-recombinase triggered short RNA hairpin directed against Jph2 (Hcn4:shJph2). The investigators reported that in vivo Hcn4:shJph2 mice exhibited evidence for sinoatrial node (SAN) dysfunction, characterized by inappropriate sinus tachycardia, a mildly increased sinus node recovery time, and a blunted response to adrenergic stimulation. Isolated Hcn4:shJph2 SAN myocytes demonstrated a higher frequency of spontaneous  $Ca^{2+}$  release events ( $Ca^{2+}$  sparks), indicating an increased  $Ca^{2+}$  leak from the SR and increased RyR2 opening, similar to what was observed during Jph2 knockout in atrial and ventricular working myocardium. In the Hcn4:shJph2 SAN, this, however, was associated with a significantly smaller  $Ca^{2+}$  spark amplitude compared with controls, resulting in functional uncoupling between the  $Ca^{2+}$  clock and the membrane clock components of the SAN pacemaker

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system and leading to highly irregular SAN APs, frequent SAN exit blocks and SAN pauses. Although there was no change in mRNA expression of NCX1 in Hcn4:shJph2 mice compared with control mice,  $I_{NCX}$  was reduced by ~50% in Hcn4:shJph2 nodal cells compared with control cells at -60 mV, that is, within the range of diastolic depolarization potentials where LRCs boost the late phase of membrane depolarization providing coupling between pacemaker clocks. These results highlight an important role of Jph2 in cardiac pacemaking, via the regulation of  $Ca^{2+}$  clock cycling, including LCR periodicity and robustness, as well as in supporting functional coupling and synchronization between the components of the coupled-clock system. It, however, remains unknown whether Jph2 organizes pacemaker proteins within distinct macromolecular complexes, by positioning them in the regions of clocks' coupling, and serves as a structural stabilizer of the pacemaker dyads through dual sarcolemmal and SR membrane anchoring domains.

Pacemaker cells are widely distributed throughout the entire intercaval region of the right atrium and comprise an extensive distributed system of atrial primary and subsidiary pacemakers (the atrial pacemaker complex), which includes, but extends well beyond, an anatomically defined SAN and atrioventricular node (AVN). Though the subsidiary pacemakers can provide a relatively regular rhythm, they are characterized by a slower resting heart, slower exertional heart rates, a prolonged postpacing recovery time, and an increased beat-to-beat heart rate variability. Although being bradycardic and overdrive suppressed by the faster SAN, subsidiary atrial pacemakers can contribute to the development of atrial tachycardia in pathological conditions.

The mechanism of subsidiary pacemaker automaticity is believed to be similar to that of primary pacemakers, however, the proportion between the components of the coupled-clock system, that is,  $I_f$  and LCR-mediated  $I_{NCX}$ , in the development of diastolic depolarization was proposed to significantly vary throughout the cells that comprise the atrial pacemaker complex.<sup>3</sup> Particularly, the crucial role of the  $Ca^{2+}$  clock in spontaneous automaticity has been highlighted in the subsidiary pacemakers, especially under sympathetic stimulation in the setting of altered SAN activity. This, indeed, was shown by Yang et al<sup>4</sup> in this issue of *JACC: Basic to Translational Science*. The investigators used the previously characterized Hcn4:shJph2 mice to model junctional ectopic tachycardia (JET) via a combination of hyperthermia (38°C), sympathetic stimulation (intra-peritoneal injection with isoproterenol and caffeine),

and mechanical stretch (internal jugular injection of a normal saline bolus), the risk factors for the development of postoperative JET in children. JET represents a common complication following surgery for congenital heart disease, significantly contributing to postoperative morbidity and mortality in children. JET originates from the AVN, the underlying mechanisms are poorly understood, and most of the common antiarrhythmic drugs showed low efficacy in suppressing JET.

Yang et al<sup>4</sup> demonstrated that modeling JET in Hcn4:shJph2 mice resulted in 100% inducibility rate compared with 0% in tamoxifen-treated control mice (Hcn4:WT mice). As in their previous paper,<sup>2</sup> in the Hcn4:shJph2 JET model, the investigators observed the suppressed SAN activity and significantly up-regulated function of the  $Ca^{2+}$  clock, associated with augmented frequency of spontaneous  $Ca^{2+}$  release events and APs. Importantly,  $I_f$  blocker ivabradine failed to reduce the increased  $Ca^{2+}$  release events and suppress AVN-driven JET in Hcn4:shJph2 mice, whereas it slowed the SAN rhythm down by 40% in control mice. By contrast, EL20, a novel tetracaine-derivative, rapidly terminated tachycardia in vivo and significantly reduced the increased  $Ca^{2+}$  leak in Hcn4:shJph2 JET mice. Surprisingly, EL20 did not affect  $Ca^{2+}$  signaling and spontaneous beating frequency in control mice. These findings indicate possible distinct roles of voltage and  $Ca^{2+}$  clocks in the mechanism of automaticity in primary vs subsidiary pacemakers that could be further exaggerated in the settings of pathophysiological remodeling associated with hyperactive RyR2, including genetic mutations, chronic pressure overload, oxidative stress, inflammation, etc. However, it is unclear why Jph2 demonstrates different effects in the SAN vs AVN, suppressing automaticity in primary pacemakers while enhancing it in subsidiary pacemaker cells.

The observed results might be specifically relevant to enhanced atrial ectopy and the development of atrial tachyarrhythmias associated with distinct anatomical locations that are targeted clinically for ablation, including both pulmonary veins and non-pulmonary vein foci. Indeed, these regions of arrhythmogenic atrial ectopy coincide with the distribution of HCN4-positive subsidiary pacemakers. Furthermore, in atrial fibrillation patients, enhanced atrial ectopy was linked to leaky RyR2 and NCX-mediated automaticity. RyR2 stabilizers, such as dantrolene and tetracaine, have shown a high antiarrhythmic efficacy in suppressing atrial fibrillation in various animal models. Similarly, EL20 has been shown to decrease arrhythmia burden in a murine

model of catecholaminergic polymorphic ventricular tachycardia by reducing the diastolic leak of SR-stored  $Ca^{2+}$  by selective inhibition of RyR2 channels.<sup>5</sup> Whether EL20 is effective against atrial arrhythmogenesis requires additional studies.

Finally, EL20 is effective at a dosing range of 1 to 2  $\mu\text{g}/\text{kg}$ , which is significantly lower than those for tetracaine (0.5  $\text{mg}/\text{kg}$ ) and dantrolene (10  $\text{mg}/\text{kg}$ ). EL20 does not affect contractility and relatively short half-life to potentially offset concerns of possible toxicity, which indeed was found in comparison with flecainide, a clinically available, potent, though likely indirect, RyR2 blocker. Yang et al<sup>4</sup> found that in vivo flecainide administration (1.5 to 3  $\mu\text{g}/\text{kg}$ ) was associated with high toxicity, while failing to convert JET in Hcn4:shJph2 mice. Further studies are needed to determine the efficacy of EL20, as well as similar

RyR2-selective blockers, in the treatment of supra-ventricular arrhythmias.

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