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REVIEW

The Initiation of the Heart Beat

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During a normal lifetime, the heart may beat over 2 billion times, but the mechanisms by which the heart beats are initiated remain a subject of intense investigation. Since the discovery of a pacemaker current (I_f) in 1978, multiple studies have shown that rhythmic changes in membrane voltage (the "membrane voltage clock") underlie the mechanisms of automaticity. The I_f is a depolarization current activated during hyperpolarization. Therefore, when the cardiac cells recover, the I_f is activated and slowly depolarizes the cell membrane, leading to the onset of action potential. Recent studies, however, suggest that increased intracellular Ca (Ca_i) induced by spontaneous rhythmic sarcoplasmic reticulum Ca release (the "calcium clock") is also jointly responsible for the initiation of the heart beat. Elevated Ca_i activates another ionic current (the sodium–calcium exchanger current or I_{NCX}), leading to spontaneous phase 4 depolarization. Under normal conditions, both clocks are needed to initiate the heart beat. Malfunction of the clocks is associated with sinus node dysfunction in heart failure and atrial fibrillation. More studies are needed to determine how both clocks work together to initiate heart beat under normal and disease conditions. (*Circ J* 2010; **74:** 221–225)

Key Words: Electrophysiology; Sick sinus syndrome; Sinoatrial node

The title of this review came from a book written by Dr Dennis Noble in 1979.[1] At that time, changes in the membrane ionic currents were thought to be responsible for the spontaneous diastolic depolarization that initiates the heart beat from the sinoatrial node (SAN). More recent data, however, show that spontaneous Ca release from the sarcoplasmic reticulum (SR) is also an important mechanism of SAN automaticity. These 2 mechanisms (membrane ionic current and spontaneous SR Ca release) work together to generate sinus rhythm.

The Voltage Clock and SAN Automaticity

Lakatta, Maltsev and their collaborators used the terms "sarcolemma voltage clock" and "subsarcolemmal Ca clock" to describe the mechanisms of SAN automaticity.[2–4] The voltage clock is formed by voltage-sensitive membrane currents, such as the hyperpolarization-activated pacemaker current (I_f).[5,6] This current is also referred to as a "funny" current because, unlike the majority of voltage-sensitive currents, it is activated by hyperpolarization (from −40/−50 mV to −100/−110 mV) rather than depolarization. The I_f is a mixed Na^+–K^+ inward current modulated by the autonomic nervous system through cAMP.[7] Sympathetic stimulation (isoproterenol) increases the I_f whereas parasympathetic stimulation (acetylcholine) reduces it.[8] These findings suggest that I_f is responsible for heart rate control by the autonomic nervous system. At the end of the action potential, the I_f is activated and depolarizes the sarcolemmal membrane.[9,10] The depolarization activates $I_{Ca,L}$. Ca entry from the $I_{Ca,L}$ activates the cardiac ryanodine receptor (RyR2) to initiate SR Ca release (Ca-induced Ca release), leading to contraction of the heart, a process known as EC coupling. The intracellular Ca (Ca_i) is then pumped back into the SR by SR Ca-ATPase (SERCA2a) and completes this Ca cycle. In addition to I_f, multiple time- and voltage-dependent ionic currents have been identified in cardiac pacemaker cells as contributing to diastolic depolarization. These currents include (but are not limited to) $I_{Ca\text{-}L}$, $I_{Ca\text{-}T}$, I_{ST}, and various types of delayed rectifier K currents.[11] Many of these membrane ionic currents are known to respond to β-adrenergic stimulation and all contribute to the regulation of SAN automaticity by changing the membrane potential. However, many of these currents, such as $I_{Ca\text{-}L}$, $I_{Ca\text{-}T}$, also result in increased Ca_i. As will be discussed, increased Ca_i may further contribute to the SAN automaticity.

The Calcium Clock and SAN Automaticity

The I_f is not the only depolarizing current active in late phase 3 or phase 4 of the action potential. Another important ionic current that can depolarize the cell is the sodium–calcium exchanger current (I_{NCX}). In its forward mode, the I_{NCX} exchanges 3 extracellular Na^+ with 1 intracellular Ca^{2+}, resulting in a net intracellular charge gain. This electrogenic current is active during late phase 3 and phase 4 because the decline in Ca_i outlasts the SAN action potential duration. It is

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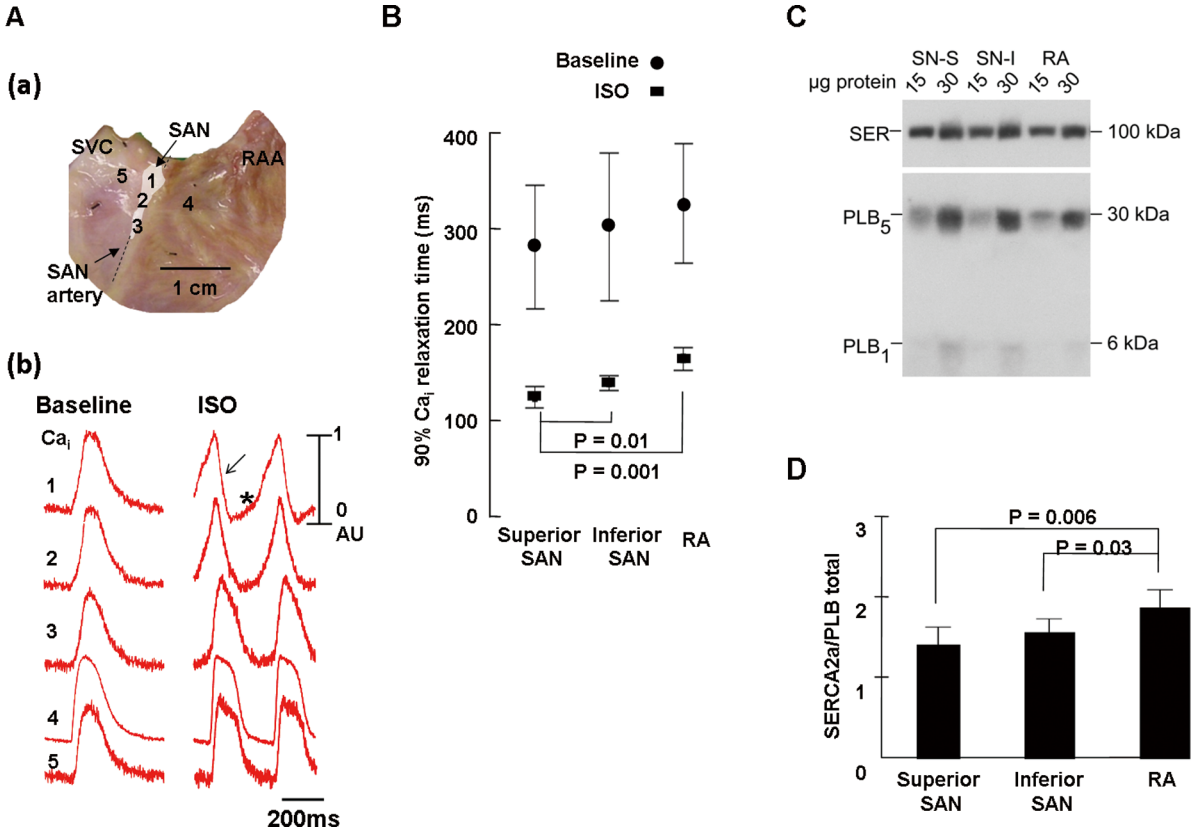

Figure 1. Differences in the Ca$_i$ physiology of the SAN and RA. (**A**) (**a**) Canine RA preparation viewed from the epicardial surface. The Ca$_i$ tracings from the superior (**1**), middle (**2**) and inferior (**3**) SAN, and from the anterior (**4**) and posterior RA (**5**) are shown in (**b**). Panel (**b**) shows the Ca$_i$ tracings at baseline (**Left**) and during 1 μmol/L ISO (**Right**). The earliest activation sites are the inferior (**3**) and superior SAN (**1**) during baseline and ISO infusion, respectively. Arrow indicates rapid reduction of Ca$_i$ at the superior SAN, followed by rapid onset of late diastolic Ca$_i$ elevation (*). (**B**) Comparison of 90% Ca$_i$ relaxation time among the superior and inferior SAN and the RA at baseline and during ISO infusion. (**C**) Western blot analyses of SERCA2a (SER) and PLB at the superior SAN (SN-S), inferior SAN (SN-I) and RA. SERCA2a and PLB are detected by anti-SERCA2a monoclonal antibody, 2A7A1, and anti-PLB monoclonal antibody, 1F1, respectively. (**D**) SERCA2a/PLB ratio of all preparations analyzed. (Reproduced from Joung et al[37] with permission.) ISO, isoproterenol; PLB, phospholamban; RA, right atrium; SAN, sinoatrial node; SERCA2a, SR Ca-ATPase; SR, sarcoplasmic reticulum.

known that under pathological conditions, spontaneous SR Ca release can induce delayed afterdepolarizations and triggered activity through I_{NCX} activation.[12] Because ryanodine receptors control the SR Ca release,[13] ryanodine-receptor-based therapy may be effective for cardiac arrhythmias.[14] Recent studies, however, showed that I_{NCX} also participates in normal pacemaker activity.[15–20] Data from Lakatta and Maltsev's laboratory showed that spontaneous rhythmic Ca^{2+} release from the SR in SAN cells results in Ca$_i$ elevation, which in turn activates I_{NCX} and leads to membrane depolarization.[21–27] This process is highly regulated by cAMP and the autonomic nervous system.[27] In the latter study, the authors show that a high basal level of cAMP and increased cAMP-dependent protein kinase A (PKA) activity in isolated rabbit SAN cells are obligatory for the occurrence of spontaneous, basal SR Ca release and spontaneous beating.[27] According to their findings, sympathetic stimulation accelerates heart rate by PKA-dependent phosphorylation of proteins that regulate Ca$_i$ balance and spontaneous SR Ca cycling. These proteins include phospholamban (PLB), a SR membrane protein regulator of SERCA2a, and L-type Ca channels. The PKA-dependent phosphorylation of these proteins controls the phase and size of subsarcolemmal SR Ca release. The resultant I_{NCX} is crucial for both basal and reserve cardiac pacemaker function.

Importance of the Calcium Clock in an Intact SAN

Many of the elegant works on automaticity were performed in isolated SAN cells, but the SAN is a complex structure and many factors interact to ensure the initiation of the heart beat.[28–32] Activation maps in the intact canine right atrium (RA) have shown that the SAN impulse origin is multicentric,[33] and sympathetic stimulation predictably results in a cranial (superior) shift of the pacemaking site in humans[34] and dogs.[35] Based on evidence from isolated SAN myocytes, late diastolic Ca$_i$ elevation prior to the membrane action potential upstroke is a key signature of pacemaking by the Ca^{2+} clock. Salama's laboratory has developed methods of simultaneously mapping the membrane potential and Ca$_i$.[36] We adapted their techniques to study the role of the Ca and voltage clocks on SAN automaticity and on the mechanisms of pacemaker hierarchy in the intact SAN.[37] **Figure 1A(a)**

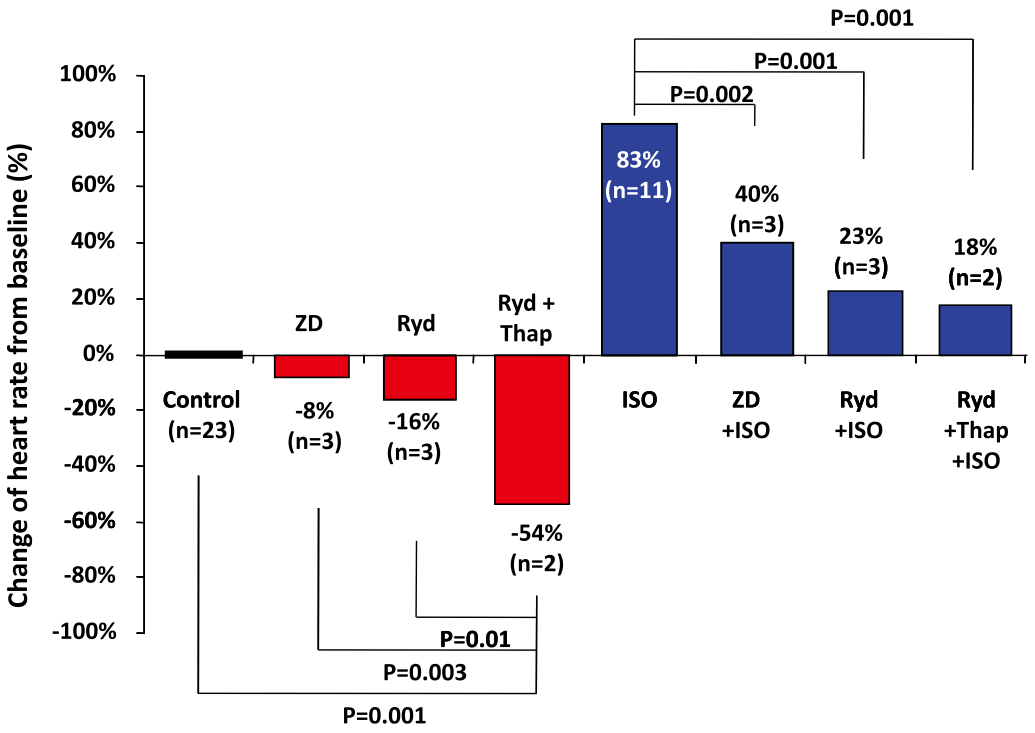

Figure 2. Spontaneous heart rate of canine RA preparation depends upon both Ca-related mechanisms and the I_f. Bars show the change in heart rate (% from baseline) induced by different pharmacological interventions. The red bars show the changes during 3 μmol/L ZD 7288 (ZD), 3 μmol/L ryanodine (Ryd), and 10 μmol/L ryanodine plus 200 nmol/L thapsigargin (Thap) infusion without ISO; blue bars show the changes during 1 μmol/L ISO infusion. (Reproduced from Joung et al[37] with permission.) I_f, pacemaker current; ISO, isoproterenol; RA, right atrium.

shows the canine RA preparation used in our study in which we found that at baseline, spontaneous diastolic SR Ca release, which is manifested by a late diastolic Ca_i elevation, occurred in only a small percentage of the preparations. The absence of late diastolic Ca_i elevation does not rule out Ca sparks, as the sensitivity of the camera may not be sufficient to detect small changes in Ca_i. However, after isoproterenol infusion, robust late diastolic Ca_i elevation was observed in all preparations (**Figure 1A(b)**). The late diastolic Ca_i elevation was associated with a superior shift of the leading pacemaker site. Most importantly, the site of the maximum slope of the late diastolic Ca_i elevation always co-localized with the leading pacemaking site, suggesting a shift in which the voltage clock now lagged behind the Ca clock. In addition, we also showed that the morphology of the Ca_i transient at the leading pacemaker site was characterized by a rapid downslope (**Figure 1A(b)**), suggesting rapid Ca_i uptake followed by rapid diastolic Ca_i elevation (**Figure 1A(b)**). **Figure 1B** shows the quantitative analysis of this phenomenon. The 90% Ca_i relaxation time was shortest at the superior SAN (leading pacemaker site), followed by the inferior SAN and RA. Various pharmacological interventions in the RA preparation confirm the involvement of late diastolic Ca_i elevation in controlling heart rate. As expected, ryanodine, which blocks ryanodine receptors at relatively high dosages, caused a dose-dependent suppression of SAN activity, and impaired isoproterenol-induced late diastolic Ca_i elevation. The combination of ryanodine and thapsigargin also suppressed SAN activity, and the impaired isoproterenol-induced late diastolic Ca_i elevation. In contrast, the I_f blocker, ZD 7288 (3 μmol/L), did not prevent the late diastolic Ca_i elevation in the superior SAN. This observation indicates a strong association between late diastolic Ca_i elevation and pacemaking during β-adrenergic stimulation, and provides new insight into pacemaker hierarchy in the canine RA.[37] **Figure 2** summarizes the heart rate responses to various pharmacological interventions in the RA preparation. At baseline and during isoproterenol infusion, the most profound heart rate reduction was induced by a combination of thapsigargin and ryanodine, which completely inhibited SR function. In contrast, the I_f current blocker ZD7288 had only limited effects on heart rate.

Heterogeneity of Membrane Proteins and SAN Function

The late diastolic Ca_i elevation in the SAN was not homogeneous. Rather, with increasing doses of isoproterenol, the earliest Ca_i elevation, the fastest Ca_i reuptake and the highest slope all occurred at the superior SAN. The inferior SAN and the other RA sites showed much less response to isoproterenol.[37] A possible explanation is that there is differential protein expression at these sites. The key protein regulator of SR Ca uptake is PLB, which inhibits SERCA2a in the dephosphorylated state and reverses its inhibition of SERCA2a in the phosphorylated state during β-adrenergic stimulation.[38] To study the distribution of these 2 proteins in the SAN, Joung et al[37] performed quantitative Western blot analysis of the expression ratio of SERCA2a/PLB in the superior and inferior parts of the SAN and RA in 4 dogs (**Figure 1C**).

Their results showed that there was a significantly lower expression ratio at SAN sites than at RA sites (Figure 1D) and of the 2 SAN sites, the superior SAN appeared to have a lower ratio than the inferior SAN, but the difference was not statistically significant. These changes suggest that during isoproterenol infusion, more PLB molecules may be phosphorylated to allow more ERCA2a molecules to be disinhibited in the SAN than in the RA, so more robust Ca uptake occurs in the SAN than in the RA during isoproterenol infusion.

Bradycardia in Patients With Deficient I_f or Abnormal Calcium Handling

Abnormalities of either the membrane clock or the Ca clock can result in bradycardia. One example is a mutation of the hyperpolarization-activated nucleotide-gated channel (HCN4), which is part of the channels that carry I_f. Mutations of the HCN4 causes familial bradycardia.[39–41] However, the bradycardia caused by HCN4 mutations may be entirely asymptomatic. Although all the mutations cause bradycardia, the heart rate response to exercise may be either suboptimal with a maximum rate of 100 beats/min[40] or entirely normal with maximum rates >150 beats/min.[41] The presence of a normal heart rate response cannot be explained by the HCN4 mutation in those patients. Therefore, although these findings support the importance of I_f in generating SAN automaticity in humans, exercise-induced heart rate acceleration may be caused by a different mechanism, the most likely being increased activity of the Ca clock because of phosphorylation of PLB and by increased activity of $I_{Ca,L}$ and RyR2 during sympathetic stimulation.

Normal SR Ca release also depends on a complex formed by calsequestrin (CSQ), RyR2, junctin and triadin.[42] Mutations of RyR2 and CSQ increase SR Ca release and cause catecholaminergic polymorphic ventricular tachycardia (CPVT).[43–45] Although tachycardia is the dominant symptomatic phenotype, it is interesting to note that patients with CPVT also exhibit significant bradycardia.[46] The association of bradycardia with Ca handling abnormalities is consistent with the hypothesis that the Ca clock is important in the mechanism of SAN automaticity. However, further investigation is needed to establish a causal relationship between the Ca clock and bradycardia in patients with CPVT.

SAN Dysfunction in Common Diseases

Common diseases, such as heart failure and atrial fibrillation, may be associated with significant SAN dysfunction.[47,48] Malfunction of both the membrane voltage clock and the Ca clock might be present in both of these common diseases. Zicha et al reported that downregulation of HCN4 expression contributes to heart failure-induced sinus node dysfunction, and upregulation of atrial HCN4 may help to promote atrial arrhythmia formation.[49] Heart failure is also known to be associated with significant abnormalities of Ca_i regulation.[50,51] It is likely that abnormalities of both clocks are responsible for SAN dysfunction in heart failure. Atrial fibrillation is also associated with a downregulation of I_f in canine models.[52] Joung el al[53] recently performed an optical mapping study of the SAN in the intact RA from dogs with pacing-induced atrial fibrillation. They found that SAN dysfunction in atrial fibrillation is associated with Ca clock malfunction, characterized by unresponsiveness to isoproterenol and caffeine, and downregulation of RyR2 in the SAN, which together with their previous study,[37] shows that as in heart failure, SAN dysfunction in atrial fibrillation is also associated with malfunction of both the membrane and Ca clock.

Summary

Multiple time- and voltage-dependent ionic currents have been identified in cardiac pacemaker cells and they contribute to SAN automaticity. Some of these currents, such as $I_{Ca\text{-}L}$, also promote late diastolic Ca elevation and acceleration of the sinus rate by the Ca clock mechanism. Therefore, the sarcolemma membrane voltage clock and the subsarcolemmal Ca clock are interdependent and act synergistically to generate normal sinus rhythm. Genetic mutations that affect a single clock do not always result in severe symptomatic bradycardia. Rather, many patients with reduced I_f are asymptomatic and can increase their heart rate to >150 beats/min during exercise. However, in common diseases such as atrial fibrillation and heart failure, both clocks are usually impaired. In these conditions, significant SAN dysfunction can occur as a complication of the disease. We conclude that both the membrane voltage clock and the Ca clock are important in the initiation of the heart beat, and simultaneous malfunction of both clocks leads to abnormal SAN function.

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