The sinoatrial node (SAN) is the normal pacemaker of the heart. During a human lifetime it must initiate approximately 2 billion heartbeats and coordinate the cardiovascular response to our physiological and emotional demands. Disease of the SAN is common, and one of the leading indications for electronic pacemaker implantation. Advances in understanding the genetics and molecular mechanisms determining normal SAN function, and of the pathways controlling remodeling are revealing SAN disease to be heterogeneous. We review the contemporary concepts of SAN function, heart rate adaptation and SAN disease from the molecular level to clinical application. (Circ J 2014; 78: 1272–1282)

Key Words: Arrhythmia; Atrial fibrillation; Remodeling; Sick sinus syndrome; Sinoatrial node

The normal pacemaker of the heart is the sinoatrial node (SAN). It is a highly specialized structure with distinct embryology, histology, electrophysiology and ion channel expression.1 Sinoatrial node disease (SND) is common but poorly understood and the only treatment is palliation by implantation of an electronic pacemaker. We will review advances in the understanding of the genetics, molecular pacemaker mechanisms and structure of the SAN and discuss how these have led to a parallel evolution of concepts of heart rate (HR) adaptation, pathophysiology of SND and potential new treatments.

Structure and Function of the SAN

Embryology
During the development of the heart, the genetic program directs the development of contractile, rapidly conducting myocardial segments (atria and ventricles) and the slow conducting cardiac conduction system (CCS; Figure 1).2 In humans, the “default” cardiac development pathway is directed by the cardiac homeobox gene, Nkx-2.5, to make contractile myocardium; repression of this program allows the specialization and localization of the CCS.3 During the development of the caudal (venous) pole of the heart, Nkx-2.5 is repressed.2 The transcription factor Tbx18 directs development of the SAN body (the central SAN) and Tbx3 imposes a pacemaker program on this area and along the developing CCS by directing activation of (among others) the HCN4 gene, and repression of (among others) high-conductance gap junction and working myocyte specific ion channel genes (eg, Connexin [Cx]40, Cx43 and SCN5A).2 Through activation of this “pacemaker program”, the caudal pole of the developing heart eventually differentiates into the sinus horns and the SAN (Figure 1).

Structure
The histologically defined SAN sits near the junction of the superior vena cava (SVC) and right atrium (RA).4 The SAN cells are small and pale, and have poorly developed sarcomeres and sarcoplastic reticulum (SR) densely packed in an area of highly fibrous connective tissue.4 Within the connective tissue the cells are single or organized into small groups of interwoven cells surrounded by a basement membrane. The most common position of the human SAN is close to the SVC, 0.1-1 mm subepicardial within the terminal atrial groove (body), extending superiorly (head) and inferiorly (tail) in parallel with the crista terminalis (CT) but there is large anatomical variation.4

The electrophysiologically defined SAN is an extensive pacemaker complex. The site of first activation may vary from a superior to inferior position along the posterior wall of the RA and there may even be multiple simultaneous leading pacemakers.5 Endocardial high-density mapping confirms a SAN pacemaker complex extending inferiorly along the CT6 and construction of an anatomically detailed model of the rabbit SAN using a combination of histology, electrical mapping and immunohistochemistry supports the view of the extensive nature of the node (Figure 1B).7 The leading pacemaker site (site of first activation) is dynamic, a phenomenon known as pacemaker shift (Figures 1C–E).5 Pacemaker shift may be a mechanism for mediating HR modulation and this might explain alterations in P-wave morphology on the surface ECG seen in response to variation in HR (Figures 1D,E).8 A hierarchy of pacing rate exists within the SAN; the superior portion gener-
Perspectives on SAN Disease

Generation of diastolic depolarization is central to SAN pacemaker activity and alteration of the phase 4 slope (ie, the rate of depolarization dV/dt) changes the HR. Pacemaker mechanisms of the SAN are complex, depending on the mutual entrainment of 2 molecular clocks: primary membrane-generated potentials (the membrane clock), and intracellular calcium dynamics (the Ca^{2+} clock, Figure 2).

The membrane clock is dependent on the interaction of the lack of a strong hyperpolarizing K^{+} current (I_{K,1}) and the presence of depolarizing Na^{+}/K^{+} currents (principally I_{f}). From hereon, large atrial muscle cells run parallel to the CT to form a preferential conduction pathway to the atrioventricular (AV) node. The precise interaction of the SAN with the surrounding muscle is not known but in the canine and human heart there is evidence of discrete superior and inferior exit pathways from the SAN with functional block zones laterally.

Normal Electrical Activity

Working myocardium has a stable negative (hyperpolarized) resting potential during phase 4 of the action potential (AP, diastole). In contrast, pacemaker tissue displays phase 4 diastolic depolarization; the cell membrane potential becomes gradually more positive to a threshold potential until an AP is triggered (Figure 2A). Generation of diastolic depolarization is central to SAN pacemaker activity and alteration of the phase 4 slope (ie, the rate of depolarization dV/dt) changes the HR. Pacemaker mechanisms of the SAN are complex, depending on the mutual entrainment of 2 molecular clocks: primary membrane-generated potentials (the membrane clock), and intracellular calcium dynamics (the Ca^{2+} clock, Figure 2).

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The molecular correlates of I_{f} are the HCN channels 1-4. Ca^{2+} contributes significantly to late diastolic depolarization by processes that are linked to, but partially independent from, I_{f}. The Ca^{2+} current is the first of these to be activated; it is a small
current but the influx of Ca\textsuperscript{2+} is amplified via Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release from ryanodine receptors (RyR) on the SR and is one mechanism of initiation of the Ca\textsuperscript{2+} clock.\textsuperscript{13} The Ca\textsuperscript{2+} clock can also initiate via spontaneous SR Ca\textsuperscript{2+} sparks.\textsuperscript{13} Unlike the global Ca\textsuperscript{2+} release seen in ventricular myocytes in response to an AP, in SAN cells this activity is seen as localized Ca\textsuperscript{2+} release manifesting as sparks or wavelets.\textsuperscript{13} The elevation of intracellular Ca\textsuperscript{2+} generates a depolarizing membrane current via the electrogenic activity of the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger, NCX1 (movement of 1 Ca\textsuperscript{2+} out of the cell for 3 Na\textsuperscript{+} into the cell),
just described that we now understand SSS as the resultant clinical presentation of heterogeneous pathological processes principally affecting the SAN and RA; for this reason, the term SND is preferable.

Electrophysiology of SND

Invasive electrophysiological testing for SND has shown little utility in predicting disease severity, requirement for pacemaker implantation or prognosis. Significantly prolonged corrected sinus node recovery time (cSNRT) and sinoatrial conduction time (SACT) in response to atrial pacing shows reasonable specificity for symptomatic SND of 88% when the 2 tests are combined. Pacemaker implantation is reasonable if significant SND is demonstrated in this manner during the investigation of syncope. The sensitivity of the tests is only 51% (cSNRT) and 54% (SACT), so the negative predictive value in assessing syncopal patients is limited. The SNRT in response to disopyramide or atropine challenge has better sensitivity than overdrive pacing and may be used when the diagnosis is equivocal, but a positive test in isolation should not be interpreted as an indication for a pacemaker.

Disease and Remodeling of the SAN

Sinus Bradycardia

The pre-eminent disease of the SAN is usually referred to as sick sinus syndrome (SSS) or tachy-brady syndrome because of the frequent coexistence of atrial fibrillation (AF). Since the late 1960s it has been described as syncope, clinically significant bradycardia, sinus pauses, sinoatrial exit block, AF and chronotropic incompetence. It is among the commonest indications for pacemaker implantation worldwide. The syndrome has generally been conceptualized as a homogeneous entity (ie, as a single “disease”), but by definition this “syndrome” is a collection of symptoms and signs rather than a cogent assessment of the underlying pathophysiology. Increasing knowledge of SAN pacemaker mechanisms and genetics as producing the inward current, $I_{\text{NCX}}$. Both the membrane and Ca$^{2+}$ clocks are modulated by cyclic adenosine monophosphate (cAMP), which mediates autonomic control at the cellular level. SAN cells are dependent on constitutively high levels of cAMP for normal function and have high expression of protein kinase A and phosphodiesterases to facilitate rapid HR variation over a large physiological range.

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**Figure 3.** Right atrial activation is altered in sinoatrial node disease. The importance of electrical remodeling is illustrated by the similarities between human idiopathic SND and SND in mice with abnormalities of the Ca$^{2+}$ clock. (A) in a patient with SND earliest activity (red) occurred at an inferior location and over a greater extent of the CT and there was conduction velocity slowing across the atrium and SAN pacemaker complex. Bipolar voltage mapping (B) demonstrates large areas of low voltage (red), multiple double potentials (blue dots) and fractionated signals (red dots), indicating slowed conduction across the pacemaker complex and adjacent RA. (Reproduced with permission from Sanders P, et al. (C) CSQ2−/− mice have an abnormal Ca$^{2+}$ clock (see Figure 2) and this results in remodeling of the SAN pacemaker complex with wide distribution and inferior shift of the leading pacemaker (black dots, basal conditions; red dots, isoprenaline; white dots, acetylcholine). (Reproduced with permission from Glukhov AV, et al.) Conduction slowing is also observed in the CSQ2−/− mice (D, white asterisk denotes pacing site). AVJ, atrio-ventricular junction; CS, coronary sinus; CSQ2−/−, homozygous calsequestrin 2 deletion; LAA, left atrial appendage; LV/RV, left/ right ventricle; WT, wild type.
underlies the disorder.\textsuperscript{28} In keeping with this, areas of low voltage scar have been demonstrated throughout the SAN pacemaker complex and RA (Figure 3B).\textsuperscript{25} However, there is also ample evidence of reverse remodeling of the SAN (ie, a return to normal function),\textsuperscript{29} which suggests the involvement of SAN electrical remodeling by processes analogous to those seen in the atrial myocardium in AF.\textsuperscript{30}

In keeping with these observations, both macroscopic electrical remodeling of the SAN pacemaker complex and molecular remodeling of key ion channels and regulatory elements has been demonstrated in response to a variety of conditions known to lead to SND.

**New Paradigm Linking HR Adaptation and SND**

Physiological electrical remodeling of the SAN has also been recently demonstrated as a new mechanism for HR adaptation.
HR changes during aging, pregnancy and exercise training are mediated by electrical remodeling of the SAN rather than purely by autonomic tone. Calcium handling proteins, including CSQ and Cask/calmodulin-dependent protein kinase II (CaMKII), are central to the Ca" clock and also implicated in SND. Therefore, in addition to pacemaking, the Ca" clock might also auto-regulate SAN function by activating CaMKII-dependent signal cascades and when strongly activated can induce electrical remodeling and SND.

**Idiopathic** SND

There are a number of rare causes of SND, but “idiopathic” SND is by far the most common description and the prevalence increases with advancing age. The normal SAN during aging without clinical SND might show a decreasing volume with fatty infiltration, but other studies have found no changes. Histological studies in the 1970s attributed SND to fibrosis and cell senescence. This is not a universal finding, even in those initial studies, SSS was associated with a histologically normal SAN and severe fibrosis was noted in the SAN of a patient with normal sinus rhythm. Furthermore, recent use of late gadolinium-enhanced cardiac magnetic resonance imaging to quantify atrial scar showed a correlation of SND with left atrial (LA) fibrosis only; the lack of association with RA fibrosis casts some doubt on the SAN fibrosis as the primary pathology in idiopathic SND.

There is evidence of electrical remodeling in the pathology of SND and age-related changes in HR. An important observation is that single isolated SAN cells from mice have a slower spontaneous firing rate with aging, and clearly this cannot be accounted for by changes during aging, and clearly this cannot be accounted for by changes in HR. In humans, detailed electrophysiological study of patients with idiopathic SND have revealed widespread conduction delay that is especially pronounced along the CT, caudal shift of the leading pacemaker and a change from a multicentric (in controls) to a unicentric site of first activation. The molecular correlate of these changes is, at least in part, ion channel remodeling, which has been extensively investigated in aging animal models of SND. Ion channel expression in the SAN is known to display temporal plasticity; for example, in the neonatal rabbit the fast Na" current (I\textsubscript{Na}) is present throughout the SAN, but in the adult it is absent from the center. With advancing age there is peripheral loss of I\textsubscript{Na}, so the area with a slow AP upstroke (ie, a typical SAN AP with Ca"-dependent rather than Na"-dependent depolarization, Figure 2) extends further into the peripheral SAN. Theoretically this could lead to exit block from the SAN and an inability to drive the surrounding atrial tissue. Decreased expression of Cx43 in the vicinity of the SAN may account for the observed increase in SAN conduction time and SAN exit block seen with aging. It has also been noted in the guinea-pig that Ca" channel expression (Cav1.2) expression declines in the SAN during aging.

**Ischemic SAN Dysfunction**

Coronary artery disease (CAD) and the subsequent sinus node artery ischemia are often cited as a cause of SND. Transient sinus bradycardia and sinus arrest are commonly seen in the acute phases of myocardial infarction but this may be in part related to altered neurological influences on the heart. There is undoubtedly an association between stable CAD and chronic SND because both are diseases of aging. However, causation has not been proven and there are no published data utilizing appropriate matched controls.

Postmortem coronary angiography of 32 patients with SND identified filling abnormalities of the SAN artery in approximately 20%, but in 52 controls with isolated AV node disease there was no identifiable disease of the SAN artery. However, postmortem angiography has significant limitations and when the nodal artery was assessed in vivo it was seldom diseased (9%) in patients presenting with significant bradycardia. Two small studies have assessed the prevalence of SAN artery disease in a group of patients with SND and clinical evidence of CAD (angina, prior inferior myocardial infarction or positive stress test). Even in this preselected group there is little evidence of a strong association between SAN artery stenosis (>50% luminal diameter) and resting HR, cSNRT or clinical evidence of SND. However, severe SAN artery stenosis (75%) was suggested to impair SAN function in 6 patients with previous inferior myocardial infarction.

Pre-emptive coronary angiography in patients with isolated SND and no angina is not indicated, but is often performed. Among patients presenting with significant bradycardia, traditional coronary risk factors remain the strongest predictor of CAD. There is little evidence for coronary investigation or intervention in patients without angina and unnecessary testing of SND patients may expose them to significant harm.

**SND Dysfunction and Atrial Arrhythmia**

SND has a well-recognized association with chronic atrial arrhythmia. As described, “idiopathic” SND is associated with an RA myopathy leading to conditions that promote the development of AF. Conversely, chronic overdrive of the SAN by a primary atrial arrhythmia leads to electrical remodeling and the development of SND. There is a caudal shift of the leading pacemaker and reduced catecholamine sensitivity in patients with AF and SND (Figure 5). Canine models of AF utilizing 20 Hz atrial pacing demonstrated increased SNRT, reduced maximal SAN rate and reduced IHR. Prolongation in SNRT has been noted in patients following electrical cardioversion of persistent AF. The 2 conditions are closely linked; in a study, the post-ablation recurrence of persistent AF was predicted by a finding of a post-cardioversion sinus pause >1,100 ms, which may be a surrogate marker for the degree of atrial remodelling.

Once again, cellular electrical remodeling is implicated in this process; atrial tachycardia pacing induces abnormalities of the Ca" clock (evidenced by altered Ca" cycling, caffeine sensitivity and RYR expression) and the membrane clock (evidenced by reduction of HCN4, HCN2 and mink expression with reduction in the I\textsubscript{i} and I\textsubscript{Ks} currents). The plasticity of this process is demonstrated by reverse remodeling of SAN dysfunction after restoration of sinus rhythm by catheter ablation. The finding of SND with atrial arrhythmia is not universal, and might be explained by a protective effect of atrial tachycardia entrance block into the SAN at faster rates.

**SND Dysfunction and HF**

Fetal bradycardia contributes a significant burden in HF, accounting for approximately 41% of in-hospital HF sudden deaths. SND has been demonstrated in HF patients: they exhibited sinus bradycardia, increased cSNRT, caudal shift of...
Former endurance athletes have an increased incidence of SND and pacemaker implantation. When training and competing, athletes display a resting sinus bradycardia; the HR of elite cyclists has been reported to be approximately 30 beats/min. Although this is usually ascribed to increased vagal tone, there is a decrease of the IHR under autonomic blockade, and thus a decrease in the intrinsic pacemaker activity of the SAN.
There is widespread electrical remodeling at the cellular level, including downregulation of HCN4 and If. This novel paradigm of HR adaptation (see above) may represent a mechanistic prodrone of the chronic SND seen in athletes.

**SAN Dysfunction and Diabetes Mellitus**

SSS has been observed in diabetic patients, as well as in the streptozotocin rat model of diabetes. SAN dysfunction in diabetes mellitus may be a consequence of microvascular dysfunction or hyperinsulinemia associated with peripheral insulin resistance (in type II diabetes mellitus). Soon after diabetes induction, streptozotocin-treated rats show a marked sinus bradycardia and the expression levels of Cx40, Cx43 and Cx45 were increased in the SAN of diabetic rats.

**Inherited SAN Dysfunction**

SND occurs in children and young adults, as well as in the elderly, though most of those cases are associated with structural heart disease. However, some have no clear structural reason for developing SSS. Numerous, uncommon gene mutations have been identified that can lead to SND; these offer insight into SAN function and add weight to the argument that alterations of ion channel expression (be it acquired remodeling or inherited) underpin SND. In addition to Mendelian inheritance described below, there is polygenic susceptibility to SND; a genome-wide association study identified polymorphisms at 21 loci, including HCN4 and Nkx-2.5 that determine the HR in healthy individuals. These were found to associate with the risk of SND and pacemaker implantation.

HCN4 is the predominant HCN isoform in the human SAN. Familial HCN4 mutations have been identified in patients with sinus bradycardia that result in a lack of channel responsiveness to cAMP (and therefore sympathetic stimulation) or a change in the channel voltage dependence leading to a reduction of If. As might be expected, these mutations can cause pre-syncope, chronicotropic incompetence and AF. However, given the importance of If in normal SAN pacemaking, the phenotype of these may be more benign than would be predicted, manifesting primarily as asymptomatic sinus bradycardia. Furthermore, transgenic mice with a conditional HCN4
Inappropriate Sinus Tachycardia (IST)

IST is a nonparoxysmal condition characterized by a rapid, regular HR and an exaggerated tachycardic response to stress. It is a diagnosis of exclusion that is incompletely understood and is difficult to treat effectively. The symptoms are independent of the severity of tachycardia and persist even after HR has been lowered. There is a high prevalence of anxiety and panic disorder among those presenting with the condition. Exaggerated response to isoproterenol has been demonstrated suggesting that the underlying pathology may be β-receptor hypersensitivity; however, the IHR is also elevated, indicating intrinsic electrical remodeling of the SAN. Modification of the HR by endocardial radiofrequency ablation has a poor outcome and is difficult to achieve because of the extensive nature of the SAN pacemaker complex. Surgical excision of the SAN has been performed for a small number of refractory cases of IST; the few available data on the long-term outcomes suggest that this invasive approach is equally ineffective. The mainstay of treatment is HR lowering with ivabradine, β-blockers or non-dihydropyridine calcium channel blockers.

Treatment of SND

The only current treatment for SND is electronic pacemaker implantation. The incidence of sudden death from SND is extremely low and has not been shown to be reduced by pacemaker implantation. Therefore pacemaker implantation should be reserved for patients who have symptoms that are attributable to, and correlate with, documented relative bradycardia. There have been concerns that atrial pacing may precipitate or worsen coexistent AF, but among patients with dual-chamber pacemakers for SND the degree of atrial pacing did not correlate with progression to AF. Furthermore, atrial pacing from alternative atrial sites (septal vs. right atrial appendage) had no effect on progression of AF. This reflects the fact that the AF is caused by widespread atrial remodeling as part of the SND pathological process rather than as a direct consequence of atrial pacing. Single-chamber atrial pacing is a reasonable strategy in patients with normal AV node function as there is no demonstrable mortality benefit from dual-chamber pacing; however, there is a significant incidence of progression to AV node disease because of widespread CCS disease and subsequent requirement for re-intervention. For this reason, the routine use of dual-chamber pacing is recommended.

Increasing knowledge of the genetics and pacemaker mechanisms of the SAN over the past decade has facilitated the investigation of gene therapy for bradycardia, the “biological pacemaker” (Figure 6). Biological pacing would circumvent some of the problems associated with electronic pacemaker implantation, such as system infections and the need for generator replacements, and provide better HR variation through autonomic modulation. Significant progress has been made, including the genesis of physiologically appropriate and responsive HRs using viral transduction of HCN channels to ventricular myocytes in large animal models of AV block and stable reprogramming of ventricular myocytes to a nodal phenotype using Tbx18. The concept has also been applied to SND by overexpression of HCN channels to accelerate pacemaker activity of the caudal SAN pacemaker complex in a model of SND. Clearly, significant challenges remain before this treatment can become a clinical reality.

Conclusions

It is over 100 years since the discovery of the SAN and the complexities of the genesis of a heartbeat are now being uncovered in detail. There is increasing evidence that electrical remodeling is an important process in SND. Current knowledge brings us to a point where tailored therapy to prevent or reverse SND, targeted at the underlying cellular processes, can begin to be envisaged.

References

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