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REVIEW

Fibrosis, Electrics and Genetics

– Perspectives on Sinoatrial Node Disease –

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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 sarcoplasmic 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 CT[6] 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**).[1] 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**).[1] A hierarchy of pacing rate exists within the SAN; the superior portion gener-

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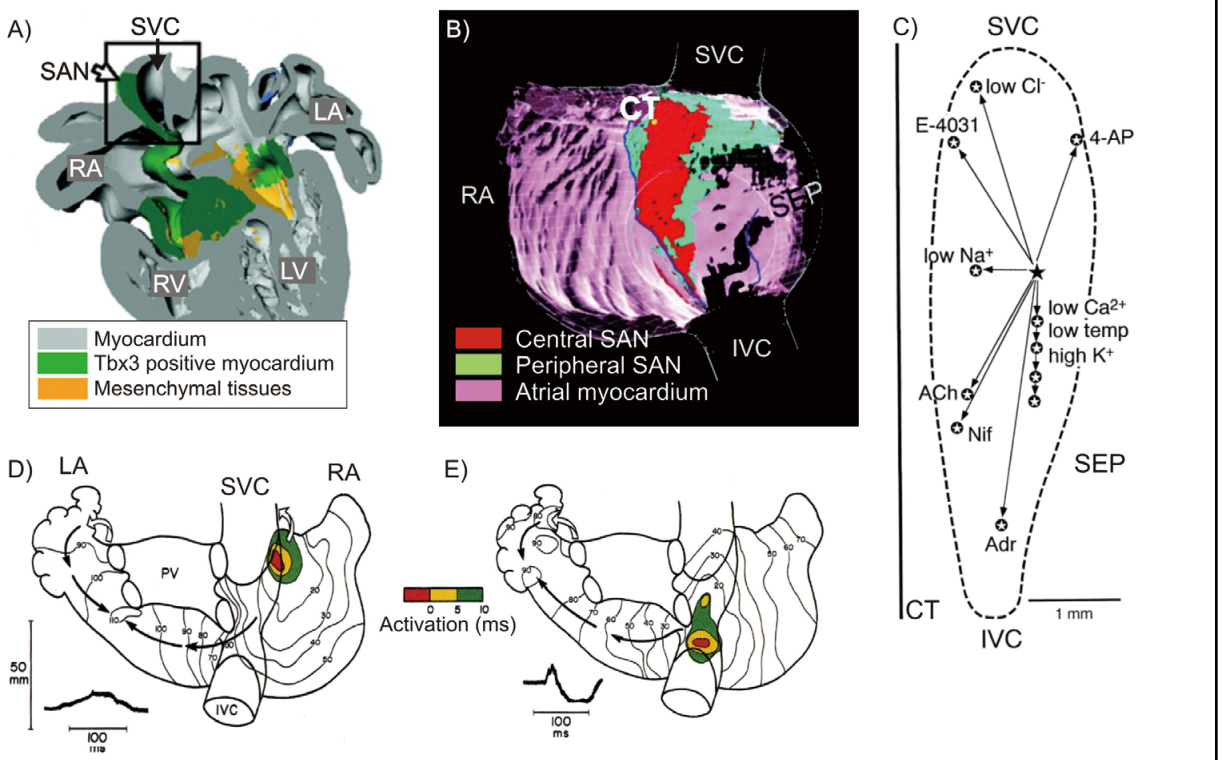

Figure 1. The sinoatrial node pacemaker complex is a dynamic and extensive structure that is defined by Tbx3-positive tissue during cardiac development. (**A**) Three-dimensional analysis of SAN formation, based on the expression of the conduction system marker Tbx3. A transverse cut through a stage 23 human embryonic heart is shown. The SAN primordium (white arrow) is in continuity with a tract of Tbx3-positive myocardium (green) of the right venous valve running posteriorly in the right atrium (RA) to the atrioventricular junction. (Reproduced with permission from Sizarov A, et al.[3]) (**B**) Endocardial view of a 3D reconstruction of a rabbit SAN is shown in a similar orientation to (**A**) for comparison. Note that the SAN shown in green and red runs along the same anatomical track as the Tbx3-positve myocardium in (**A**) from the superior vena cava (SVC) posteriorly and septal to the crista terminalis (CT) in the RA extending down to the inferior vena cava (IVC). This is based on detailed histology and known molecular markers for the SAN. (Reproduced with permission from Dobrzynski H, et al.[7]) (**C**) Functionally, within the SAN pacemaker complex there is "pacemaker shift"; the position of the normal leading pacemaker is shown by the black star; changes in response to the indicated conditions by the white stars. (Reproduced with permission from Boyett MR, et al.[95]) (**D**,**E**) Epicardial mapping of normal sinus rhythm in humans. The entire length of the SAN pacemaker complex is able to support pacemaking under basal conditions. The P-wave morphology may change if the atrial activation is altered (aVF shown inset). (Reproduced with permission from Boineau JP, et al.[5]) SEP, interatrial septum.

ates faster rates and there is generally increasing bradycardia with inferior shift of the leading pacemaker.[8]

Under most conditions the centre of the SAN is the leading pacemaker site; this area is small, less than 1% of the total volume. The central cells are archetypal SAN pacemaker cells and there is a gradual transition of cell type towards the periphery, with the appearance and intermingling of cells that are intermediate in morphology between nodal and atrial cells.[7] From hereon, large atrial muscle cells run parallel to the CT to form a preferential conduction pathway to the atrioventricular (AV) node.[9] 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.[10]

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**).[1] 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). In diastole, working myocytes are held at a hyperpolarized membrane potential by the inward rectifier current, $I_{K,1}$. This is absent in SAN cells, generating the earliest phase of diastolic depolarization. I_f is activated at these negative membrane potentials early in phase 4 (diastole) and further depolarizes the SAN cell.[1] The molecular correlates of I_f are the HCN channels 1-4.[11]

Ca^{2+} contributes significantly to late diastolic depolarization by processes that are linked to, but partially independent from, I_f.[12] The Ca^{2+} clock is dependent on a number of membrane channels and intracellular Ca^{2+} handling proteins. The T-type Ca^{2+} current is the first of these to be activated; it is a small

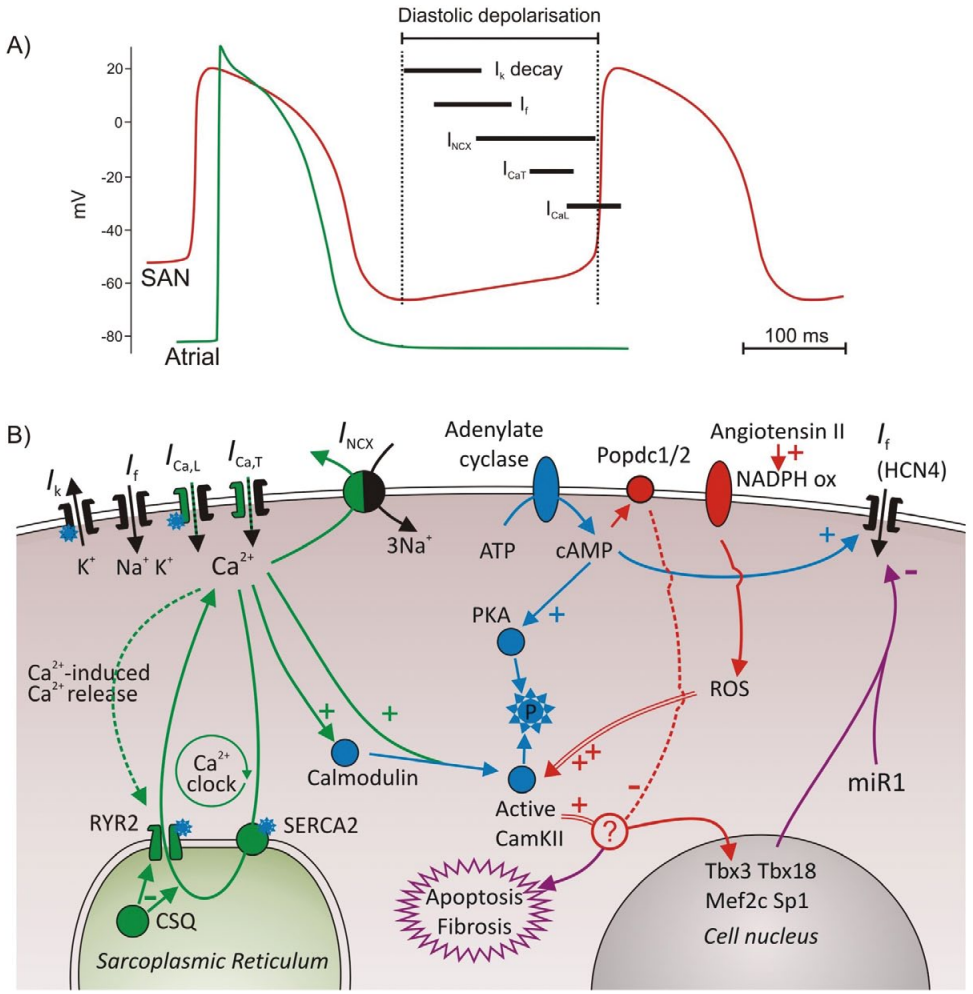

Figure 2. Molecular basis of sinoatrial node (SAN) pacemaking and disease. (**A**) Representative action potentials for the atrial myocardium (green) and central SAN (red). The temporal contributions of the main membrane and calcium currents to the diastolic depolarization are shown by the black bars. (Reproduced with permission from Monfredi O, et al.[1]) (**B**) Schematic representation of a SAN cell with central pacemaker currents, cyclic adenosine monophosphate (cAMP) and regulatory mechanisms. Pacemaking by the SAN cell depends on the membrane clock (black symbols) and the Ca^{2+} clock (green symbols) shown on the left. Points of entrainment (ie, processes that contribute to both the membrane and Ca^{2+} clocks) are shown in both black and green. The funny current (I_f) and decay of the inward rectifier current (I_k) are primarily responsible for the membrane clock and early phase 4 depolarization. Influx of Ca^{2+} through the T-type Ca^{2+} channel ($I_{Ca,T}$) and release of Ca^{2+} via ryanodine receptor 2 (RYR2) from the sarcoplasmic reticulum (SR) activates I_{NCX}. Calsequestrin (CSQ) regulates Ca^{2+} release from the SR by binding Ca^{2+} and also by inhibiting RYR2. The Ca^{2+} clock is reset by pumping of Ca^{2+} into the SR by the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA2). Normal pacemaker function is dependent on high levels of protein phosphorylation (blue symbols in center). Constitutively active adenylate cyclase produces cAMP, which binds directly to HCN channels to enhance I_f. Protein kinase A (PKA) is activated by cAMP binding, which acts in parallel with Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) to phosphorylate and modulate the function of the target ion channels (blue stars). Under normal conditions this process is regulated by high phosphodiesterase activity that acts to reduce and control phosphorylation levels (not shown). These opposing pathways facilitate rapid heart rate variation. Regulatory pathways are shown on the right (afferent limb in red, efferent limb in purple). Angiotensin II elevates reactive oxygen species (ROS) within SAN cells via activation of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase). ROS directly and permanently activates CamKII (double red arrow) independent of calmodulin and cellular Ca^{2+} by oxidation and this causes cellular apoptosis, fibrosis and down regulation of HCN4.[35] Popeye domain proteins (Popdc 1/2) bind cAMP and are protective against pathological bradycardia.[96] The transcription factors Tbx3, Tbx18, Med2c, and Sp1 and the micro-RNA miR1 are known to control HCN4 expression, but the interaction between these and the afferent pathways is not known.[33]

current but the influx of Ca^{2+} is amplified via Ca^{2+}-induced Ca^{2+} release from ryanodine receptors (RYR) on the SR and is one mechanism of initiation of the Ca^{2+} clock.[13] The Ca^{2+} clock can also initiate via spontaneous SR Ca^{2+} sparks.[13] Unlike the global Ca^{2+} release seen in ventricular myocytes in response to an AP, in SAN cells this activity is seen as localized Ca^{2+} release manifesting as sparks or wavelets.[13] The elevation of intracellular Ca^{2+} generates a depolarizing membrane current via the electrogenic activity of the Na^+/Ca^{2+} exchanger, NCX1 (movement of 1 Ca^{2+} out of the cell for 3 Na^+ into the cell),

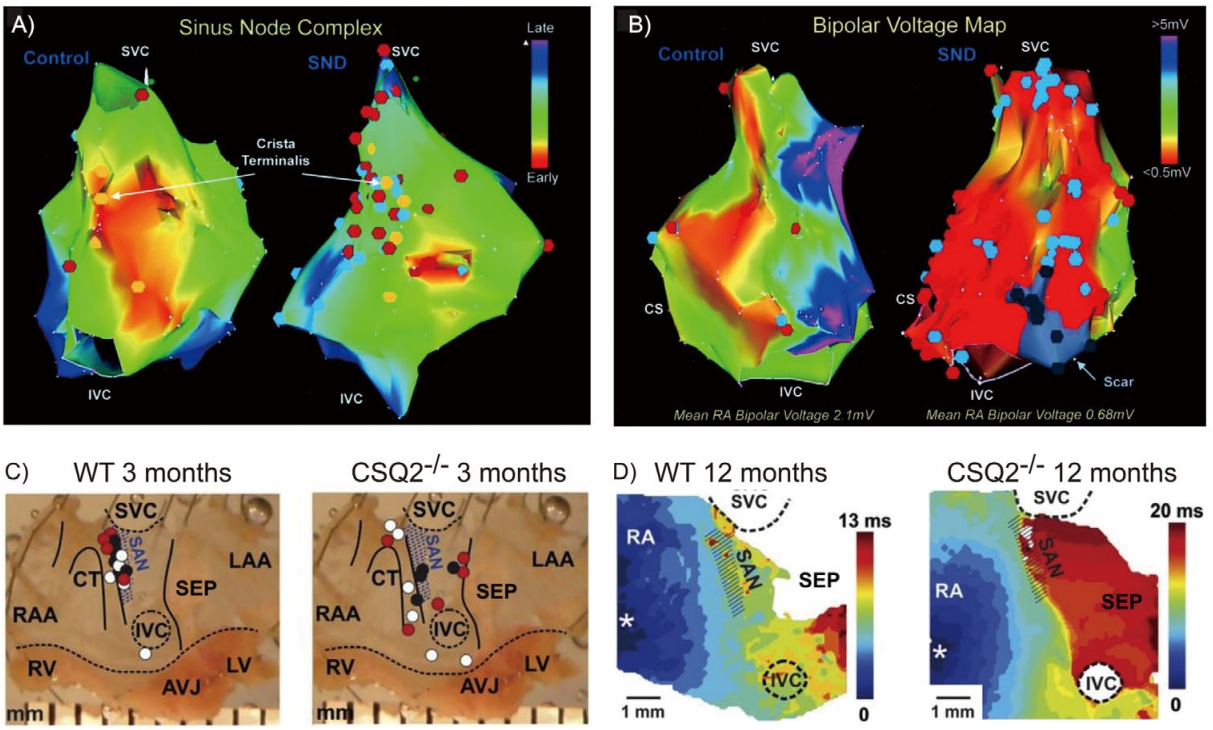

Figure 3. Right atrial activation is altered in sinoatrial node disease. The importance of electrical remodeling is illustrated by the similarities between human idiopathic SND and SND in mice with abnormalities of the Ca^{2+} clock. (**A**) in a patient with SND earliest activity (red) occurred at an 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.[25]) (**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.[34]) Conduction slowing is also observed in the $CSQ2^{-/-}$ mice (**D**, white asterisk denotes pacing site). AVJ, atrioventricular junction; CS, coronary sinus; $CSQ2^{-/-}$, homozygous calsequestrin 2 deletion; LAA, left atrial appendage; LV/RV, left/right ventricle; WT, wild type.

producing the inward current, I_{NCX}.[13] Both the membrane and Ca^{2+} clocks are modulated by cyclic adenosine monophosphate (cAMP), which mediates autonomic control at the cellular level.[14,15] 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.[16]

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.[17] It is among the commonest indications for pacemaker implantation worldwide.[18] 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 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.[19] Pacemaker implantation is reasonable if significant SND is demonstrated in this manner during the investigation of syncope.[20] The sensitivity of the tests is only 51% (cSNRT) and 54% (SACT), so the negative predictive value in assessing syncopal patients is limited.[19] 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.[21,22]

Results gained from assessment of invasive study of SAN and RA function have advanced our understanding of SND.

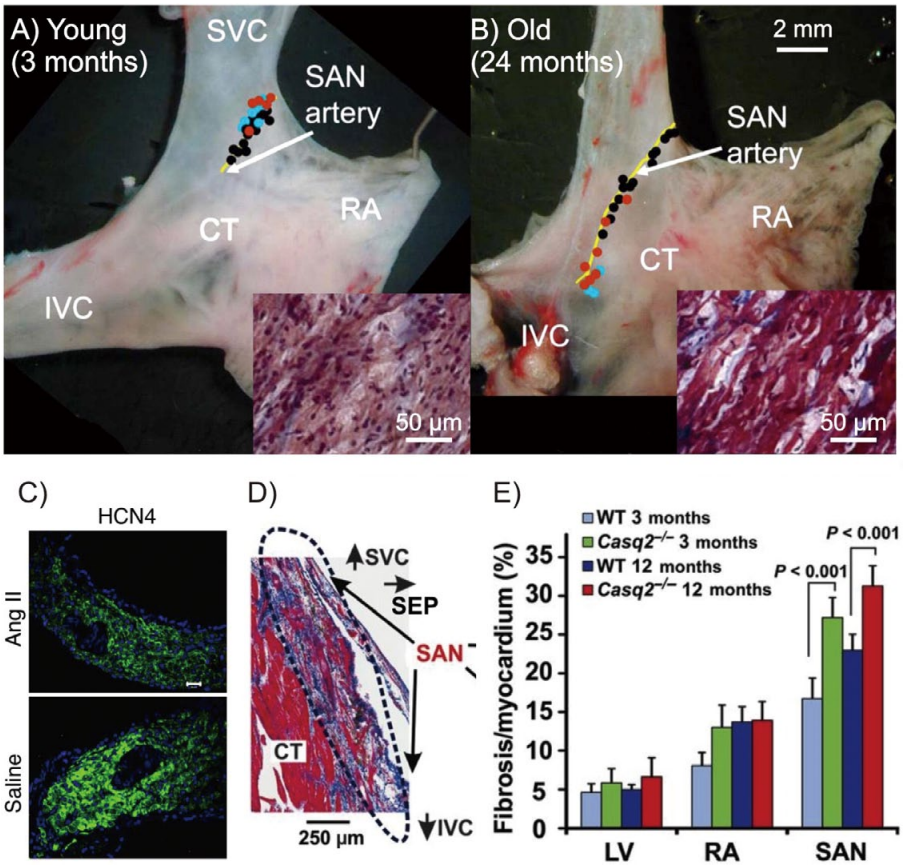

Figure 4. Fibrosis is a common, but not universal finding in sinoatrial node (SAN) remodeling. (**A**, **B**) There is a reduction in the intrinsic heart rate with age and a redistribution of the SAN pacemaker complex similar to that seen with SAN disease (SND; see **Figure 3**). (**A**) In young adult rats, pacemaker action potentials (blue and red dots) can only be recorded in a limited area near the superior vena cava (SVC) but in (**B**) 24-month-old rats (human equivalent ≈69 years) pacemaker potentials are redistributed over a large area extending inferiorly towards the inferior vena cava (IVC). Despite this remodeling, the SAN in both ages shows a similar level of fibrosis and mRNA expression of fibrotic markers (mRNA data not shown; black dots in (**A**) and (**B**) represent recordings of atrial muscle action potentials, inset panels show Masson's trichrome: red, muscle; blue, collagen; black, cell nuclei). (Reproduced with permission from Yanni J, et al.[43]) Angiotensin II (AngII) causes remodeling of the SAN by oxidizing CamIIK (see **Figure 2**). (**C**) Electrical remodeling in response to AngII is evident by reduced immunostaining (green) of HCN4. In this model there is also widespread fibrosis of the SAN (similar to that shown in **D**) and the right atrium (RA). (Reproduced with permission from Swaminathan PD, et al.[35]) Fibrosis may be detectable even when SND has a primary electrical etiology. In addition to electrical remodeling CSQ deletion causes fibrosis (**D**) that is limited to the SAN region (**E**) because the SAN Ca^{2+} clock is linked to signaling pathways causing changes in gene expression, cell death and fibrosis (see **Figure 2** for details). (Reproduced with permission from Glukhov AV, et al.[34]) Casq2$^{-/-}$, homozygous calsequestrin 2 deletion; CT, crista terminalis; LV, left ventricle; SEP, interatrial septum; WT, wild type.

Aging is associated with alterations of SAN function, causing a decrease in the intrinsic HR (IHR, ie, the HR under complete autonomic blockade), and an increase in SACT.[23] These changes are preceded by a period of detectable but clinically silent RA remodeling, particularly apparent around the region of the CT (the caudal extension of the SAN pacemaker complex), leading to conduction slowing, voltage loss and increased SNRT.[24] In the presence of clinically detectable SND, there is a change in SAN activation. There is a caudal shift of the leading pacemaker site within the SAN pacemaker complex, both in isolated SND[25] and in the presence of heart failure (HF), AF or atrial flutter (**Figure 3**).[6,26,27]

The cause of SND is often couched in terms of fibrosis and degeneration. Methodological constraints of early studies led to a belief that progressive SAN fibrosis and loss of SAN cells underlies the disorder.[28] In keeping with this, areas of low voltage scar have been demonstrated throughout the SAN pacemaker complex and RA (**Figure 3B**).[25] However, there is also ample evidence of reverse remodeling of the SAN (ie, a return to normal function),[29] which suggests the involvement of SAN electrical remodeling by processes analogous to those seen in the atrial myocardium in AF.[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,[31] pregnancy[32] and exercise training[33] are mediated by electrical remodeling of the SAN rather than purely by autonomic tone. Ca^{2+} handling proteins, including CSQ and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), are central to the Ca^{2+} clock and also implicated in SND (**Figures 2B,3C,D**).[34,35] CaMKII is also an important molecule in pro-cardiomyopathic signaling cascades[36] and when strongly activated can induce electrical remodeling and SND (**Figures 2,4C**).[35] Therefore, in addition to pacemaking, the Ca^{2+} clock might also auto-regulate SAN function by activating CaMKII-dependent signaling cascades. This explains why perturbation of intracellular Ca^{2+} in CSQ knockout mice paradoxically causes sinus bradycardia despite elevation of intracellular Ca^{2+}.[34] Numerous transcription factors, including Tbx3, as well as microRNA-1, have been reported to regulate the HR response to exercise via electrical remodeling of SAN cells.[33] This offers a unifying theory of physiological HR adaptation and the maladaptive development of SND (**Figure 2B**).

"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.[37] The normal SAN during aging without clinical SND might show a decreasing volume with fatty infiltration,[38] but other studies have found no changes.[39] Histological studies in the 1970s attributed SND to fibrosis and cell senescence.[28] This is not a universal finding (**Figure 4**); even in those initial studies, SSS was associated with a histologically normal SAN[40] and severe fibrosis was noted in the SAN of a patient with normal sinus rhythm.[28] Furthermore, recent use of late gadolinium-enhanced cardiac magnetic resonance tomography 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 SAN fibrosis as the primary pathology in idiopathic SND.[41]

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 explained by fibrosis.[31] 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 (**Figure 3**).[25] 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_{Na}) is present throughout the SAN, but in the adult it is absent from the center.[42] With advancing age there is peripheral loss of I_{Na}, so the area with a slow AP upstroke (ie, a typical SAN AP with Ca^{2+}-dependent rather than Na^+-dependent depolarization, **Figure 2**) extends further into the peripheral SAN.[43] 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.[44] It has also been noted in the guinea-pig that Ca^+ channel expression (Cav1.2) expression declines in the SAN during aging.[23]

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.[45] 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.[40,46] 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.[47] 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).[48,49] 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.[48,49] However, severe SAN artery stenosis (>75%) was suggested to impair SAN function in 6 patients with previous inferior myocardial infarction.[48]

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.[47] 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.

SAN 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.[25] 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**).[26] Canine models of AF utilizing 20 Hz atrial pacing demonstrated increased SNRT, reduced maximal SAN rate and reduced IHR.[50] Prolongation in SNRT has been noted in patients following electrical cardioversion of persistent AF.[51] The 2 conditions are closely linked; in 1 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.[52]

Once again, cellular electrical remodeling is implicated in this process; atrial tachycardia pacing induces abnormalities of the Ca^{2+} clock (evidenced by altered Ca^{2+} cycling, caffeine sensitivity and RYR expression) and the membrane clock (evidenced by reduction of *HCN4*, *HCN2* and *minK* expression with reduction in the I_f and $I_{K,s}$ currents).[53,54] The plasticity of this process is demonstrated by reverse remodeling of SAN dysfunction after restoration of sinus rhythm by catheter ablation.[29] 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.[55]

SAN Dysfunction and HF

Fatal bradycardia contributes a significant burden in HF, accounting for approximately 41% of in-hospital HF sudden deaths.[56] SND has been demonstrated in HF patients: they exhibited sinus bradycardia, increased cSNRT, caudal shift of

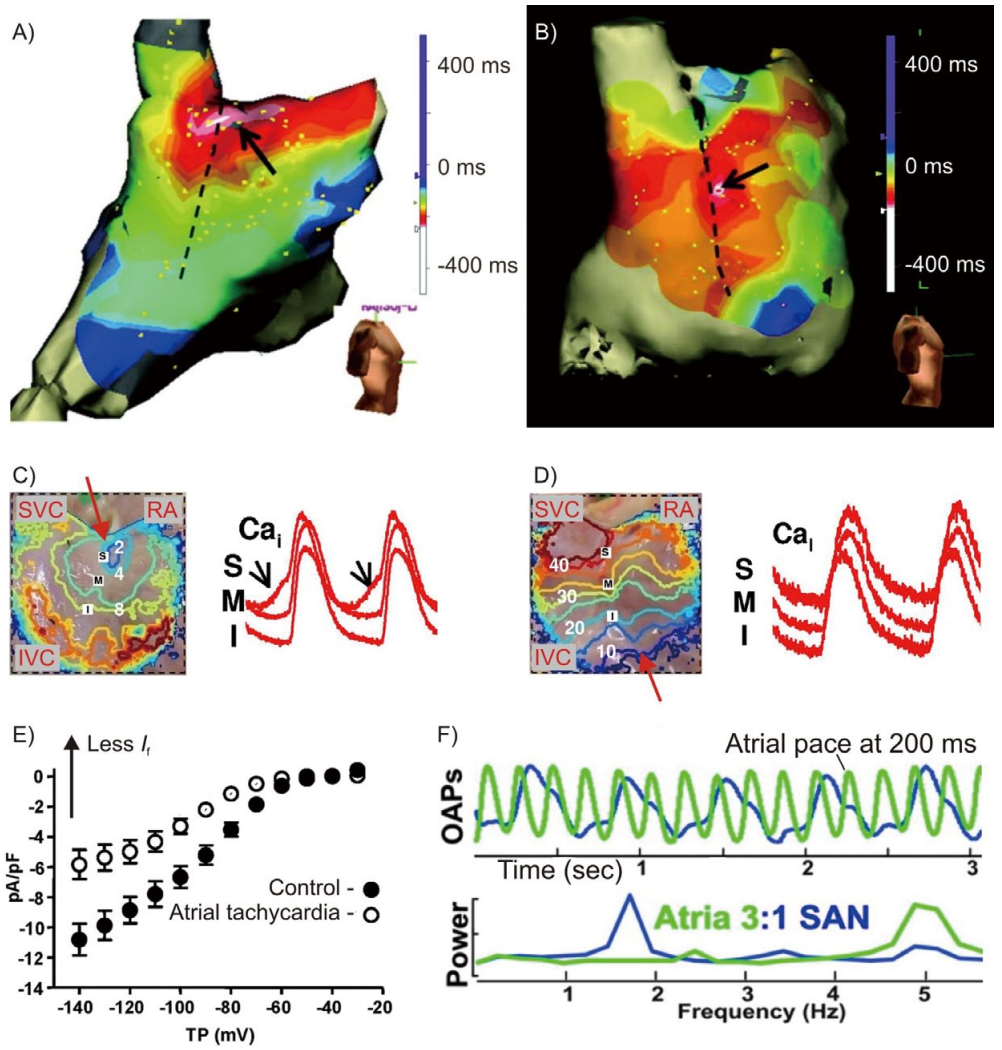

Figure 5. Atrial tachyarrhythmia causes electrical remodeling of the sinoatrial node. During sinus rhythm and isoproterenol infusion the earliest activation (black arrow) in control patients (**A**) is high on the crista terminalis near the superior vena cava (SVC), whereas in patients with atrial fibrillation (AF) and SAN disease, the earliest activation is shifted inferiorly (**B**). (Reproduced with permission from Joung B, et al.[26]) A similar phenomenon is seen in a canine model of pacing-induced AF with evidence of electrical remodeling of the SAN. Epicardial activation is shown for a control animal in (**C**), earliest activation superiorly shown by the red arrow and there is late diastolic Ca^{2+} elevation (LDCAE) in the SAN (black arrows; S, superior; M, mid; I, inferior). (Reproduced with permission from Joung B, et al.[97]) After 18 days of pacing-induced AF (**D**), there is an inferior shift of the leading pacemaker and Ca^{2+} clock dysfunction (no LDCAE). (Reproduced with permission from Joung B, et al.[97]) Remodeling of the membrane clock has also been demonstrated (**E**); there is a reduction of I_f and HCN protein in response to atrial tachycardia pacing. (Reproduced with permission from Yeh YH, et al.[54]) However, there is conduction block of atrial tachyarrhythmia into the SAN, which may impart heterogeneity on the degree of remodeling that occurs (**F**). During atrial pacing at a cycle length of 200 ms (green line), the SAN is activated at 600 ms (blue line; OAPs, optical action potentials). (Reproduced with permission from Fedorov VV, et al.[55]) IVC, inferior vena cava; pA/pF, current density (picoamperes/picofarad); TP, test potential.

the leading pacemaker, prolongation of SACT and abnormal circuitous propagation of the sinus impulse.[27] The SAN remodeling may be related to abnormal hemodynamics of the RA in HF and as such represent a RA cardiomyopathy. Consistent with this concept, SAN dysfunction has been demonstrated in association with conditions leading to RA pressure and volume overload, including dyssynchronous AV pacing, pulmonary hypertension and chronic atrial septal defect.[57–59] In a canine model of HF, cellular electrical remodeling was seen with downregulation of HCN channels in the SAN, leading to SND.[60]

SND in Endurance Athletes

Former endurance athletes have an increased incidence of SND and pacemaker implantation.[61] When training and competing, athletes display a resting sinus bradycardia; the HR of elite cyclists has been reported to be approximately 30 beats/min.[62] 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.[63]

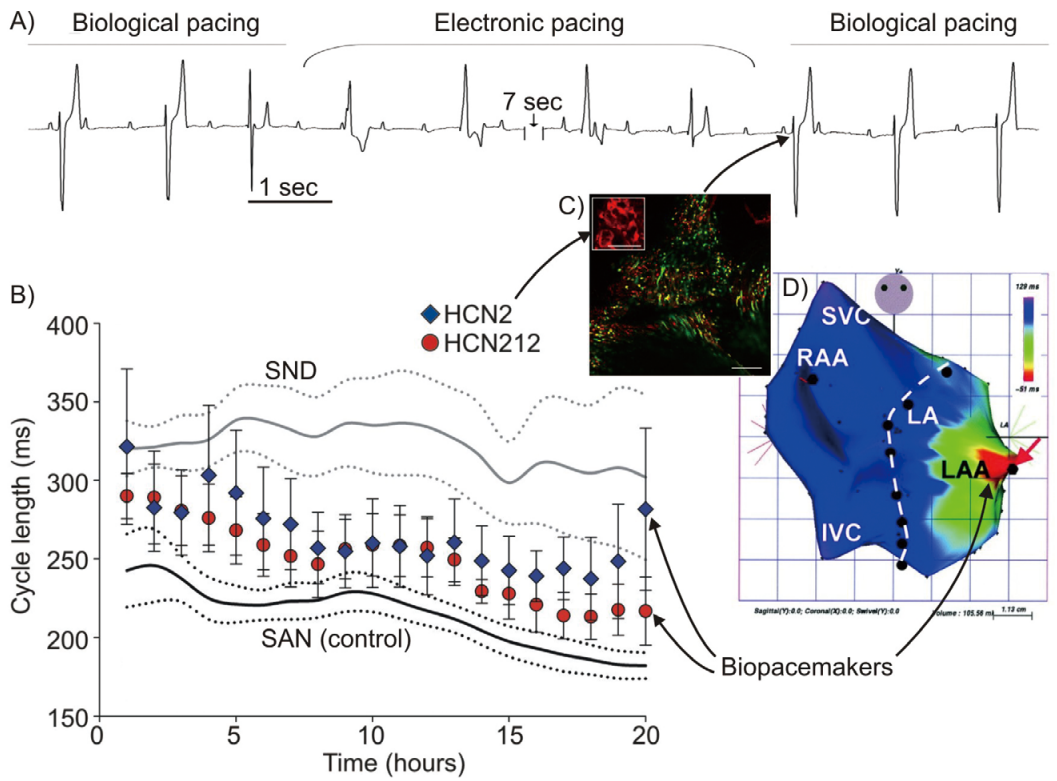

Figure 6. Biological pacemakers are emerging as a potential alternative to electronic pacemakers in cardiac conduction system disease. Extensive work has established proof of concept for biopacemaking especially in atrioventricular block; in this example (**A**), adenoviral transduction of HCN2 in the left bundle branch of dogs with atrioventricular block reduced dependence on electronic pacing. (Reproduced with permission from Bucchi A, et al.[91]) The concept has also been applied to models of SND (**B**–**D**). In (**D**), following ablation of the SAN in pigs biological pacing was induced by transduction of HCN1 (red arrow) in the left atrial appendage (LAA). (Reproduced with permission from Tse HF, et al.[98]) Subsidiary pacemaker tissue in the inferior right atrium that is active in SND (see **Figure 3**) can be harnessed as a substrate for HCN-based biopacemakers; the spontaneous pacing rate of this subsidiary pacemaker tissue (grey line, SND in **B**) is accelerated towards that of the control SAN rate (black line, SAN in **B**) by transduction of HCN channels (blue and red symbols in **B**). (Reproduced with permission from Morris GM, et al.[93]) Expression of HCN2 channels in subsidiary pacemaker tissue is shown by immunofluorescence in (**C**, expression from a bi-cistronic green fluorescence protein (GFP) vector; red, HCN2; green, GFP; scale bar 200 μm). Inset: high-power view (scale bar 20 μm). (Reproduced with permission from Morris GM, et al.[93])

There is widespread electrical remodeling at the cellular level, including downregulation of HCN4 and I_f.[33] This novel paradigm of HR adaptation (see above) may represent a mechanistic prodrome 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.[64] 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, streptozoticin-treated rats show a marked sinus bradycardia and the expression levels of Cx40, Cx43 and Cx45 were increased in the SAN of diabetic rats.[65]

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.[66] 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 polygenetic 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.[67] These were found to associate with the risk of SND and pacemaker implantation.

HCN4 is the predominant HCN isoform in the human SAN.[1] 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)[68] or a change in the channel voltage dependence leading to a reduction of I_f.[69–72] As might be expected, these mutations can cause pre-syncope,[72] chronotropic incompetence[68] and AF.[71] However, given the importance of I_f in normal SAN pacemaking, the phenotype of these may be more benign than would be predicted, manifesting primarily as asymptomatic sinus bradycardia.[69,70] Furthermore, transgenic mice with a conditional *HCN4*

knockout develop sinus pauses but are not profoundly bradycardic.[73]

The preservation of relatively normal SAN function in the presence of significant *HCN* mutations underscores the importance of the Ca^{2+} clock. During normal SAN pacemaking, there is rhythmic Ca^{2+} release via RYRs in SAN cells during late diastole (late diastolic Ca^{2+} elevation [LDCAE]).[74] Mutations of the Ca^{2+} channels and Ca^{2+} handling proteins are associated with SAN dysfunction though there is a more severe global cardiac phenotype because, unlike HCNs, these proteins are less specific to the SAN. Sinus bradycardia is a recognized feature of CPVT, an inherited arrhythmia caused by mutations of RYR or calsequestrin 2 (CASQ2).[75] Mutations of both proteins are associated with CPVT and SAN dysfunction,[76,77] and CASQ2 knockout mice have depressed SAN function with alterations of SAN Ca^{2+} cycling and release (**Figure 3**).[34]

A broad range of familial *SCN5A* mutations have been identified that cause SND as part of their phenotype.[78] The mutations may lead to a nonfunctional Na^+ channel, a change in the voltage dependence of the channel or reduced I_{Na} density.[78] Though I_{Na} is absent from the central SAN where the leading pacemaker usually arises, alterations of I_{Na} affect SAN pacing function by changing the coupling of the peripheral SAN to the surrounding atrial myocardium, increasing SAN hyperpolarization and suppressing pacing activity.[79] Abnormalities of ion channel trafficking caused by an ankyrin-B mutation causes severe SND by disrupting the membrane and Ca^{2+} clocks, with reduced expression of NCX, Na^+/K^+-ATPase, inositol triphosphate receptor 3 (IP3), and $Ca_v1.3$.[80]

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.[81] The symptoms are independent of the severity of tachycardia and persist even after HR has been lowered.[81,82] There is a high prevalence of anxiety and panic disorder among those presenting with the condition.[82] Exaggerated response to isoprenotenolol 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.[83] 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.[84] 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.[85] 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.[86] Therefore pacemaker implantation should be reserved for patients who have symptoms that are attributable to, and correlate with, documented relative bradycardia.[20] 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.[87] Furthermore, atrial pacing from alternative atrial sites (septal vs. right atrial appendage) had no effect on progression of AF.[88] This reflects the fact that the AF is caused by widespread atrial remodeling as part of the SND pathological process[25] 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.[89] 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**).[90] 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[91] and stable reprogramming of ventricular myocytes to a nodal phenotype using Tbx18.[92] 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.[93] Clearly, significant challenges remain before this treatment can become a clinical reality.[90,94]

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.

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