Pharmacologic Approach to Sinoatrial Node Dysfunction

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Abstract

The spontaneous activity of the sinus node initiates the heartbeat. Sinus node dysfunction (SND), also referred to as 'sick-sinus-syndrome', is caused by failure to generate a normal sinus node action potential. In the clinical practice, SND is generally considered as an age-related pathology, secondary to degenerative fibrosis of the heart pacemaker tissue. However, other forms of SND exist, including idiopathic primary SND showing genetic legacy and forms that are secondary to cardiovascular or systemic disease. The incidence of SND in the general population is expected to increase over the next sixty years, boosting the needs for implantation of electronic pacemakers. During the last two decades, our knowledge of sinus node physiology and of the pathophysiological mechanisms underlying SND has advanced considerably. This review will summarize the current knowledge about SND mechanisms and discuss the possibility of introducing new pharmacologic therapies for handling SND.

Keywords: Sinus Node Dysfunction; SAN; G protein-activated K^+ channels; Cav1.3; Ankyrin-B; Tertiapine-Q;

Introduction

The cardiac impulse is generated in the sinus node (SAN) by a highly-integrated mechanism involving ion channels, intracellular Ca^{2+} dynamics, membrane receptors and connexins (1; 2). Despite the intrinsic robustness of the pacemaker mechanism, SAN dysfunction (SND) constitutes a relatively common clinical condition, especially among the population over 65 (1/600, (3)). SND generally manifests as an insufficiency of the heart rate to meet the needs of the organism. SND may be differentiated into reversible (acute), or chronic symptomatic forms (4).

Acute SND is generally handled pharmacologically or by temporary transvenous or transaesophageal pacing (4; 5). In contrast, only a few options are currently available to treat chronic symptomatic SND, and to date permanent electronic pacing (PPM) by an implanted electronic pacemaker remains the primary and definitive therapy (4; 5). In this regard, symptomatic SND and heart block account for about half of the total pacemaker implantations in the U.S (6) and this is predicted to double over the next half century (7). In addition, clinical studies indicate an increasing necessity for implantation of complex electronic pacemakers (8).

In this review, we will discuss some of the most documented forms of primary and secondary SND, especially in relation to current pharmacologic management. Research over the last 20 years has considerably advanced our knowledge of SND aetiology. Several genes coding for ion channels (9-21), scaffolding proteins (22), cytoskeleton proteins (23), as well as connexins and proteins involved in cardiac development have been linked to previously unexplained forms of primary familial SND (24). Furthermore, development of animal models of primary and secondary SND, as well as *ex vivo* exploration of SANs from human hearts with history of SND have shed new light in the mechanisms of secondary forms of SND. These approaches have identified a host of novel targets for managing SND, including cardiac G protein-activated I_{KACh}/I_{Ado} (GIRK1/GIRK4) (25-29) and Ca^{2+}-activated small conductance K^+ (SK) channels (30-32). The identification of GIRK channels as potential molecular targets for SND may extend the indication of pharmacologic approaches to chronic forms of SND. More generally, it is possible that innovative molecules targeting specific SND mechanism will help manage chronic SND, which is now handled only by PPM.

Clinical Definition of SAN Dysfunction

The diagnosis of SND is based on the correlation between the patient's symptoms and ECG hallmarks (see also Supplementary Appendix 1), which provide important criteria for PPM (4; 5; 33). Historically, SND patients have been identified as having one or more of the following ECG findings (34): (i) persistent, unexpected sinus bradycardia (**Figure 1**), (ii) short periods of sinus arrest during which atrial or junctional rhythms replace normal sinus rhythm, (iii) long periods of sinus arrest in the absence of junctional rhythms, resulting in cardiac standstill, and finally (iv) episodes of sinus exit block not related to drug therapy (34; 35). This early definition of SND remains in use in current clinical guidelines (4; 5; 36). Sinus bradycardia is generally defined as a heart rate below 50 bpm (4). Sinus pauses or sinus arrest are included in the current definition of SND, particularly when manifest as 'tachycardia-bradycardia syndromes', in which sinus bradycardia, pauses or arrest follow periods of abnormal atrial tachycardia, atrial fibrillation or flutter (37). In tachycardia-bradycardia syndromes, SND can manifest as poor or sluggish return of sinus rhythm following cardioversion (38) (**Supplementary Figure 1**). Another hallmark of SND is chronotropic incompetence, defined as the inability of heart rate to attain 80% of the expected heart rate during exercise (39). Symptomatic SND carries invalidating symptoms that can impact quality of life (36). One of the most common symptoms of SND is syncope, which is present in

about half of SND patients (5; 40). While asymptomatic bradycardia is not associated with adverse outcomes, patients with untreated symptomatic SND have high risk of deterioration to cardiovascular events including atrial fibrillation (41), heart failure (42) and systemic thromboembolism (5; 40). Age-dependent SND and chronotropic incompetence are associated with an increased risk of cardiovascular death and overall mortality (4).

SAN Pacemaking: general overview

The SAN is a highly-complex, heterogeneous tissue (1). Surprisingly, pacemaker cells do not constitute the predominant cell type in the SAN. Early studies of SAN tissue indicated that atrial myocytes (43) and fibroblasts (44) are important constituents of SAN structure and integrative properties. More recently, RNA sequencing indicates that the SAN is composed of atrial myocytes, adipocytes, epithelial cells, fibroblasts, vascular endothelial cells, macrophages and neurons (45). In addition to heterogeneous cellular composition, pacemaker cells within the SAN are poorly-coupled electrically (46). This low inter-cellular conductance is due to high expression of Cx45 and low or absent expression of Cx43 in pacemaker cells (46-48). Finally, the SAN region includes specific non-conductive structures and redundant impulse propagation pathways (29; 49) that help ensure proper intra-sinus and sinus-to-atria conduction (50; 51).

Normal pacemaking depends on a unique action potential (AP) profile of SAN pacemaker cells. Importantly, the SAN AP undergoes a spontaneous diastolic depolarization phase driving the membrane voltage from the end of the repolarization to the threshold of the following action potential (**Figure 2**). Catecholamines positively regulate the slope of the diastolic depolarization via activation of ß-adrenergic receptors (ßARs). The adrenergic activation stimulates the synthesis of cAMP, that positively regulates the activity of several ion channels of the plasma membrane, and the intracellular ryanodine receptors/Ca^{2+}-release channels (RyR2) embedded in the sarcoplasmic reticulum (SR) (2; 52) (**Figure 2**). In antagonism with ßARs, muscarinic type 2 receptors (M2Rs) and adenosine type 1 (A1Rs) receptors decrease pacemaking by promoting down-regulation of intracellular cAMP and by inducing opening of G protein activated K^+ channels (GIRK1/GIRK4) underlying the I_{KACh} / I_{KAdo} current (53). Current understanding of the generation of the heartbeat is best summarized by the "coupled-clock" model of pacemaking (52), stating that generation of diastolic depolarization is the result of a functional interplay between activity of ion channels at the plasma membrane and local diastolic RyR2–dependent Ca^{2+} release, which is coupled to the diastolic depolarization via activation of the Na^+/Ca^{2+} exchanger (NCX1) (2; 52; 54). Different ion channels of the plasma membrane contribute to the generation and the regulation of the diastolic depolarization and will be discussed here briefly.

The hyperpolarization-activated "funny" current I_f, is activated at the end of the repolarization phase of the action potential and supplies inward current throughout the range of the diastolic depolarization (55). Catecholamines shift I_f activation curve to more positive voltages, while acetylcholine induces a negative shift. These opposing effects are explained by the direct sensitivity of f-channels to cAMP (56), which increases the probability of channel opening at a given voltage (57). F- channels are encoded by the hyperpolarization activated cyclic nucleotide gated channel (HCN) family, which comprises four distinct isoforms HCN1-HCN4. However, in the SAN, the predominant isoform is HCN4, accounting for 80% of the total HCN mRNAs (58). Moreover, HCN1 protein is almost exclusively expressed in the human SAN rather than in atrial myocardium (59). Both T- and L-type Ca^{2+} channels are expressed in SAN pacemaker cells. Cav3.1 and Cav3.2 mRNAs are expressed in the SAN however, the predominant functional T-type isoform in the adult SAN is Cav3.1 (60). In spite of this low availability, Cav3.1 knockout mice (*Cav3.1*$^{-/-}$) present with moderate SAN rate reduction (-10%) and prolonged atrioventricular (PR) conduction interval (60). SAN pacemaker cells concomitantly express two distinct L-type Ca^{2+}

channel isoforms, Cav1.3 and Cav1.2 (61). Cav1.3-mediated I_{CaL} is characterized by a more negative threshold for activation than Cav1.2-mediated I_{CaL} (-45 mV for Cav1.3- vs -25 mV for Cav1.2-mediated I_{CaL}, respectively) (61). Under basal conditions, *Cav1.3*$^{-/-}$ pacemaker cells show erratic generation of automaticity, a lack of the linear phase of the diastolic depolarization (62) and a strong reduction of the total inward diastolic current compared to control littermates (26; 62).

Voltage-gated "neuronal" (n)Nav Na^+ channels underling the sino-atrial TTX-sensitive Na^+ current (I_{NaTTX}) have been shown to contribute to pacemaking and intra-nodal conduction in the mouse, rabbit and human SAN (63-66). Several nNav1 isoforms have been proposed to contribute to sino-atrial I_{NaTTX}. While nNav1.1 and nNav1.3 have been proposed to contribute to I_{NaTTX} in mouse and rabbit (63), nNav1.6 appears to play a dominant role in human intra-nodal conduction and SND (66). The "cardiac" (c)Nav1.5 isoform underlies the TTX-resistant I_{Na} in the mouse SAN, but its contribution to excitability may be limited to intra-nodal or nodal-atrial impulse conduction. Consistent with this hypothesis, mice haplo-insufficient for Nav1.5 present with atrio-ventricular and intra-ventricular conduction defects, rather than with SND.

Ion channels of the transient receptor potential channels family (TRP) contribute to SAN activity (67-69). TRPC channels contribute to store-operated Ca^{2+} entry in pacemaker cells (69). TRPM7 channels contribute to pacemaking by regulating HCN4 channels in the sinus and atrioventricular node (70). TRPM4 channels contribute to the basal beating rate of SAN pacemaker cells (67).

More recently, members of Ca^{2+} activated K^+ channel family (K_{Ca}) have been linked to pacemaking in mouse and rabbit SAN. K_{Ca} have been subdivided into Big (71), Intermediate (31) and Small (32; 72) conductance K^+ channels. These channels have differential sensitivity to Ca^{2+}. Big K_{Ca} (BK) are primarily voltage-dependent with positive regulation by Ca^{2+} (73). In contrast, Intermediate and Small KCa (IK and SK, respectively) are voltage-independent and highly sensitive to Ca^{2+}(31). Such sensitivity to Ca^{2+} is mediated by the association with calmodulin (CaM) (73) and at least for SK channels coupling with L-type Ca^{2+} channels has been demonstrated (74; 75). SK isoforms have been identified with different sensitivity to the specific inhibitor apamin (SK1, SK2 and SK3) (73). All three SK isoforms are expressed in the SAN (32).

Familial Primary SAN Disease and Associated Conduction Defects

While SAN disease and SND are often associated with aging or with different cardiovascular pathologies such as heart failure (76; 77), work over the past two decades has identified familial forms of SND (24; 78). In fact, congenital forms of SAN disease have been instrumental in providing molecular insights into the critical and non-redundant molecular pathways underlying SAN automaticity and signaling. Multiple ion channels and ion channel subunits are now associated with SAN disease.

HCN4 gene (*HCN4*) variants are linked with human asymptomatic bradycardia or SAN disease (9-12). Mechanistically, variants have been associated with altered channel membrane targeting, aberrant ion channel activation (10) or conductance (12), as well as dysfunction in channel regulation by cyclic nucleotides (9; 13). Of note, human *HCN4* variants are linked with other forms of cardiovascular disease beyond the SAN demonstrating the key role of this ion channel (11). Beyond *HCN4*, human variants in *SCN5A* are linked with SND (79). Loss-of-function of the *SCN5A* gene encoding for Nav1.5 accounts for 5% of total incidence of primary conduction system dysfunction including SND in humans (21; 80). *SCN5A* variants are linked with familial SND, bradycardia, conduction disorders, as well as atrioventricular block (24; 78). However, similar to variants in other key cardiac ion channels, *SCN5A* variants are linked with other forms of atrial and ventricular arrhythmia.

While not as robustly expressed in the working myocardium as Cav1.2, Cav1.3 channels (*CACNA1D*) are highly expressed in the SAN (61; 81-83) (see also preceding section). While rare,

human *CACNA1D* variants that alter channel activity are linked with human SND (14; 15). Cav1.3 channels have been linked also to neonatal complete heart block related to lupus (84). Mechanistically, heart block has been explained by the presence of maternal auto-antibodies against Cav1.3 channels (85; 86). In addition to Cav1.3, T-type Cav3.1 channels (encoded by *CACNA1G*) have also been linked with human bradycardia and heart block related to neonatal lupus (84; 87).

TRPM4 (encoded by *TRPM4*), also called transient receptor potential cation channel subfamily M member 4, is a nonselective calcium-regulated channel expressed in the SAN and atria. *TRPM4* variants have been widely linked with a number of human cardiac phenotypes including sinus bradycardia likely through modulation of the SAN cell membrane potential (88; 89).

Alterations in a number of SAN and atrial accessory and calcium regulatory proteins have been associated with human SND. Ankyrin-B, encoded by *ANK2*, is a cytoskeletal adaptor protein that associates with a host of cardiac ion channels, transporters, signaling molecules, and structural proteins. Consistent with animal models lacking ankyrin-B, humans harboring specific variants in *ANK2* may display bradycardia and conduction defects (22). Mechanistically, ankyrin-B dysfunction alters multiple critical SAN proteins, including Cav1.3 and NCX1, resulting in aberrant diastolic depolarization. It is likely that specific genetic and environmental factors influence SND penetrance and severity in the human population based on the degree of ankyrin-B loss-of-function and potential secondary variants or environmental factors. Similar to ankyrin-B, cardiac caveolin-3 (*CAV3*) variants, while linked with ventricular arrhythmia, have also shown to be associated with bradycardia (90). Mechanistically, caveolin-3 variants may impact multiple ionic currents to influence SAN node automaticity.

More recently, an exciting set of papers have linked human variants in proteins that regulate heterotrimeric G proteins to SND. To date, both *GNB5* and *GBN2* variants are linked with sinus bradycardia/SND (17; 18). Similar to other genes outlined in this review, individuals harboring variants may display additional non-cardiac phenotypes including cognitive disorders. Mechanistically, these variants may impact the activity of I_{KACh}, inducing current gain-of-function and consequent sinus bradycardia and atrioventricular block. Consistent with this hypothesis, mutation in *KCNJ3* and *KCNJ5* inducing gain-of-function of GIRK1 and GIRK4 were shown to be linked with familial SAN disease (*KCNJ3* encodes GIRK1; *KCNJ5* encodes GIRK4) (16; 91).

Dysfunction in cardiac calcium regulatory proteins are linked with familial SND. Calsequestrin 2 (*CASQ2*) is a calcium-binding protein that also serves a critical role in regulating local calcium release and automaticity. The cardiac ryanodine receptor (*RYR2*) is a central regulatory node responsible for excitation-contraction coupling. Variants in either *RYR2* or *CASQ2* are associated with bradycardia and potentially fatal arrhythmias in response to catecholamines (92-94). In particular, catecholaminergic polymorphic ventricular tachycardia (CPVT) is a life threatening familial ventricular arrhythmia associated with mutations in *CASQ2* or *RYR2* (95). *RYR2*-associated CPVT is characterized by increased Ca^{2+} release from the SR at rest and under adrenergic activation (95). However, some CPVT patients carrying mutations in *RYR2* present also with moderate sinus bradycardia (96; 97). Bradycardia has been explained by tonic Ca^{2+}-dependent inactivation of I_{CaL} and reduction of basal SR Ca^{2+} load in SAN pacemaker cells (96; 97).

Additional forms of inherited SND involve a host of cardiac proteins with a range of functions in the myocyte. For example, human variants in *MYH6* that encodes cardiac muscle myosin are linked with SND (as well as other non-SAN electrical and structural phenotypes) (98). Like myosin, lamins A and C have multiple roles for cardiac function. Encoded by *LMNA*, lamin A variants are associated with sinus bradycardia and conduction system disorders (99). Finally, variants in a number of other genes including *SHOX2* (100), a transcription factor involved in differentiation of the SAN, have been linked with cardiac SAN and/or atrioventricular block phenotypes.

It is important to note that like other forms of familial human disease, penetrance and disease severity will depend on both secondary genetic, environment, and social factors. Thus,

caution should always be utilized when interpreting genetic information from individual variant carriers.

Genetic mouse models of primary SND

A wide variety of genetically modified mouse models have been generated to study SAN disease. Among the first mouse models of SND were those involving knockout of ion channels important for SAN membrane excitability. Early studies found that *Cav1.3*$^{-/-}$ mice showed congenital deafness, bradycardia and irregular heart rate (101). Further work has shown that *Cav1.3*$^{-/-}$ mice constitute a model of the SAN Dysfunction and Deafness (SANDD) syndrome, which is characterized by sinus bradycardia and atrioventricular conduction dysfunction (14). Indeed, *Cav1.3*$^{-/-}$ mice have pronounced sinus bradycardia, associated with sinus pauses, atrial fibrillation and flutter, and 2nd –degree atrioventricular block (26; 61; 102). Global knockout of *Hcn4* prevents proper development of the SAN and conduction system resulting in embryonic lethality (103). Inducible *Hcn4* knockout mice have also been generated and found to have either mild SND with sinus pauses (104) or severe bradycardia and conduction system defects incompatible with life (105). Knockout of *Hcn1* induces bradycardia and SND in the mouse (106). Mice carrying genetically silenced I_f conductance show sinus bradycardia and SND associated with 2nd-degree atrioventricular block and ventricular arrhythmia (25). On the other hand, mice lacking cAMP-dependent regulation of HCN4 show moderate sinus bradycardia, consistently to what observed in individuals carrying cAMP regulation defective HCN4 (9; 13).

Consistent with the clinical findings, mice heterozygous for Nav1.5 display bradycardia, sinus exit block, and reduced excitability of pacemaker cells (107). Proteins important for intracellular ion homeostasis have also been explored using genetic mouse models. Atrial-specific knockout of the NCX1 (sarcolipin-Cre crossbred with NCX1 floxed mouse) display sinus arrest, burst pacemaking and junctional escape rhythm (108). In a similar vein, knockout of the SR Ca^{2+} buffer calsequestrin disrupted SAN Ca^{2+} handling leading to irregular pacemaking and SND, consistent with observations in patients (109).

There is a growing number of mouse models with SND and defects in unconventional ion channels and accessory proteins not typically invoked when discussing SAN pacemaking. For example, the background K^+ channel TREK-1 has recently emerged as a novel determinant of SAN excitability and pacemaking. Specifically, mice with cardiac specific ablation of TREK-1 show bradycardia with frequent sinus pause (110). At the cell level, TREK-1-deficient SAN pacemaker cells show a depolarized maximum diastolic potential and altered firing rate. Similarly, several TREK-1 interacting partners have been linked to SND in mice, including the cytoskeletal protein βIV-spectrin (111) and members of the Popeye-domain containing (POPDC) family (112). Mouse models of TRPM4 related SND have been generated. Although the TRPM4 knockout (*TRPM4*$^{-/-}$) mouse shows no difference in heart rhythm compared to wild-type counterparts, pharmacological inhibition of TRPM4 slows spontaneous beating in wild-type but not *TRPM4*$^{-/-}$ atria (113). Furthermore, TRPM4 mice show more frequent episodes of sinus pauses and prominent conduction block compared to wild-type counterparts (114). TRPM7 is a divalent channel-kinase abundantly expressed in mouse and human heart. Global as well as sinus/atrioventricular node-restricted knockout of TRPM7 slows the diastolic depolarization in pacemaker cells and induces bouts of sinus pause and conduction block (70). As discussed above, Ankyrin-B is an adapter protein important for proper membrane localization of multiple ion channels and transporters important for normal SAN excitability and pacemaking. Human variants in ANK2 have been linked to a complex arrhythmia syndrome including severe SND requiring pacemaker implantation (22). Consistent with the human phenotype, mice heterozygous for ankyrin-B display severe bradycardia and enhanced resting heart rate variability compared to wild-type littermates (22).

Mouse models have been instrumental in studying the link between dysregulation of gene expression and SND. Notably, mice deficient for the transcription factor Tbx3 show defects in development of the SAN and of the conduction system. They show also SND characterized by bradycardia and sinus pause presenting in the embryonic stage (115). The cardiac homeobox transcription factor Nkx2-5 has been studied using an atrial-specific knockout model, which shows hyperplasia of the working myocardium and conduction system, resulting in a broad spectrum of arrhythmias including bradycardia and conduction block and perinatal lethality (116). Mechanistically, Nkx2-5 deficiency was shown to activate Notch signaling and enhance myocyte proliferation early in development. Interestingly, Notch signaling is also activated with injury and a genetic mouse model has been used to study effects of transient Notch activation in the atria with observations of reduced *SCN5A* expression and structural remodeling of the SAN, resulting in sinus bradycardia and sinus pause (117). Genetic mouse models have also elucidated a role for signaling through the multifunctional Ca^{2+}/calmodulin-dependent kinase II in SND in the setting of neurohumoral dysregulation. Specifically, transgenic mice overexpressing a CaMKII inhibitory peptide (AC3-I) were found to be resistant to development of fibrosis and sinus pause following chronic angiotensin II infusion (118). As new experimental models have been developed and applied to study SAN function and disease, so too have a host of computational models of pacemaker activity (See Supplementary Appendix 2). These models will prove important for understanding SND as the discovery of new mechanisms progresses.

Secondary forms of SND in humans and animal models

Secondary forms of SND account for the majority of patients. Indeed, the incidence of SND correlates with age, co-morbidities such as hypertension, diabetes, presence of cardiovascular disease and elevated plasma concentrations of cystatin-c and natriuretic peptide (7). However, current guidelines generally make distinction between secondary forms of SND associated with systemic disease, cardiovascular disease and drug intoxication (4). In relation to systemic conditions, SND can be a secondary manifestation of endocrine disease such as hypothyroidism and diabetes, inflammatory or rheumatologic disorders, plasma ionic imbalance and infectious disorders (4; 76).

Abnormal input of the autonomic nervous system is a potential cause underlying SND. In some patients, intrinsic SND can be worsened by autonomic nervous system imbalance (119). In addition, increased vagal input (hypervagotonia) has been proposed to constitute the direct cause of SND manifestations (119). Clinical studies have established a correlation between endurance athletic training and an increased risk of bradyarrhythmia, atrioventricular block (120) and atrial fibrillation (121), requiring PPM (122). Both hypervagotonia and intrinsic remodelling of SAN ion channels expression have been proposed as mechanisms of SND in endurance athletes. I_f current and HCN4 are downregulated in animal models of athletic training (123; 124). In addition, the heart rate of endurance athletes shows reduced sensitivity to the I_f blocker ivabradine, suggesting reduced HCN expression (123). It is thus possible that both changes in the sympathovagal balance and intrinsic remodeling of ion channels in SAN pacemaker cells contribute to bradyarrhythmia induced by athletic training.

Often SND secondary to cardiovascular disease is associated with atrial tachyarrhythmia and atrial fibrillation. In early studies, SND was present in 5% of patients affected by atrial fibrillation for less than one year. However, SND incidence increased up to 45% in patients suffering from atrial fibrillation for 10 years or more (38). Interestingly, in a canine model of atrial fibrillation induced by atrial tachypacing, SND was explained by a reduction in the expression of I_f -channels (125). This mechanism can account for SND associated with chronic or persistent atrial fibrillation. However, since a significant number of patients present with progressively worsening

SND, in which association with atrial fibrillation and conduction dysfunction constitute late co-morbidities, it is an attractive hypothesis that early pharmacologic handling of SND may prevent associated arrhythmias. Ischemic disease can also lead to SND. Typical examples of ischemic conditions favoring SND are acute stenosis or thrombosis of the SAN artery (126) and myocardial infarction (127). As discussed above, heart failure is a major provider of secondary SND. Several mechanistic aspects of how myocardial heart failure leads to SND are still to be identified. However, in rabbit (128), canine (129) and mouse (130) models of heart failure, reduction of pacemaker activity has been attributed to down-regulation of I_f current and its predominant SAN isoform HCN4, with consequent SND.

Myocardial infarction can often degenerate into heart failure and consequently, bring secondary SND forms. A study combining myocardial infarction with diabetic condition showed an increase in oxidized CaMKII, apoptosis of pacemaker cells, SND and mortality in wild-type mice. These effects were prevented in knockin mice expressing oxidation-resistant CaMKII, in which paired methionines in the CaMKII regulatory domain mutated to valines (131). Oxydation of CaMKII could thus constitute an important mechanism in SND secondary to myocardial infarction and neurohumoral dysregulation potentially leading to heart failure (118).

To date, several mechanisms have been proposed to account for age-related SND. The reader can find a detailed discussion about age related SND in a recent review by Peters et al. (132). Interestingly, similar to other secondary forms of SND, decline of pacemaker activity with ageing is attributed to intrinsic remodeling of the SAN structure and expression of ion channels involved in automaticity. Decrease in the intrinsic electrical coupling due to progressive tissue fibrosis has been proposed as a primary factor in age-related SND (see Csepe et al. for review (133)). However, some individuals having high degree of SAN fibrosis can be under normal sinus rhythm (134). In addition, recent work demonstrated slowed intrinsic pacemaker activity and reduced densities in I_f, I_{CaL} and I_{CaT} in SAN pacemaker cells of bradycardic aged mice (135). It is thus possible that both structural and ionic factors contribute to age-related SND in a patient's dependent way.

Pharmacological approaches to SND in the current clinical practice

There is a wide panel of potentially usable drugs for acute bradycardia and SND however, the most widely used are catecholaminergic agonists like isoproterenol, atropine, aminophylline and theophylline. Isoproterenol is a β-selective agonist devoid of vasoconstrictional effects. Isoproterenol has shown some positive effects in ameliorating heart rate in patients with bradycardia. However, because isoproterenol administration can induce also supraventricular tachycardia (136), its use is recommended only for intra-hospital management of acute SND or electrophysiological evaluation of SND (4). Since β-adrenergic activation augments the myocardial oxygen demand accompanied by coronary vasoconstriction, its use is not indicated in SND associated with cardiac ischemic disease (4). Other catecholaminergic agonists such as epinephrine or dopamine present more complex effects because of their mixed α– and β– receptor activation (137). Use of catecholamines is thus indicated only under intra-hospital and haemodynamic monitoring conditions (4). Atropine is a well-known inhibitor of muscarinic receptors. Clinical studies have showed heart rate improvement in patients with acute bradycardia and SND, including secondary to myocardial infarction (138; 139). Atropine is also used for diagnostic evaluation of SND (140; 141). However, some adverse effects have been reported with atropine, including tachycardia and psychotic states (138; 141). Theophylline and aminophylline belong to the pharmaceutical class of methylxanthines. Theophylline in particular, is probably the most widely used drug to handle SND under out of hospital settings. The beneficial effect of methylxanthines

in improving heart rate is attributed to their blocking action on adenosine receptors (142), which could make these drugs suitable in handling bradycardia in post-transplantation hearts (143; 144) and following spinal cord injury (145). In a limited clinical study, theophylline has also showed improvement of heart rate, without significant adverse effects, which prevented PPM (146).

Drug intoxication constitutes an additional challenge for pharmacologic handling of bradycardia attributable to SND (4). Frontline treatment of SND following intoxication with β-blockers, Ca^{2+} channels blockers and digitalis (digoxin) exist. Intoxication with β-blockers can be handled by administration of glucagon, which stimulates hepatic adenylate cyclase leading to increased glycolysis. No systematic clinical studies including cohorts of patients is available to validate the use of glucagon for SND secondary to intoxication with β-blockers (147). However, available data support the concept of antagonizing the effect of β-blockers and Ca^{2+} channels blockers using glucagon or insulin to improve heart rate in patients' SND symptoms (148). Finally, intravenous bolus of calcium-gluconate can be used to oppose to the effects of Ca^{2+} channels blockers such as verapamil or amlodipine (4; 148). Digoxin intoxication induces complex arrhythmia pattern and bradyarhythmia (149). Digoxin is a poorly dialyzable drug consequently, treatment of SND secondary to glycoside intoxication is applied using specific anti-digoxin antibodies (150). Immunotherapy against digoxin intoxication is effective in improving heart rate, with relative low associated mortality (151).

New pharmacologic targets to SND in animal models

Recently, it has also been possible to test new potential pharmacologic approaches to SND by targeting GIRK channels. Our group has tested this hypothesis by crossing *Girk4*$^{-/-}$ mice with mice in which I_f conductance has been genetically silenced (25). Genetic ablation of I_{KACh} effectively rescued SND and atrioventricular dysfunction in these double-mutant mice and prevented also SND-associated ventricular arrhythmia (25). Interestingly, isoproterenol was effective in elevating the heart rate of these mice, but failed to correct SND hallmarks such as sinus pauses and atrioventricular dysfunction (25), suggesting that direct inhibition of I_{KACh} could be a more effective strategy than catecholaminergic stimulation. Furthermore, genetic ablation of I_{KACh} was effective in rescuing SND and atrioventricular dysfunction also in SANDD *Cav1.3*$^{-/-}$ mice (26). Rescuing of SND is observed also by direct inhibition of I_{KACh} by tertiapine-Q (**Figure 3**). This phenomenon of "compensatory" genetic or pharmacologic targeting of I_{KACh} can provide new therapeutic option for SND in the future (26; 152) (see also Supplementary Appendix 3).

While no primary forms of SND linked to K_{Ca} channels have been described to date, evidence indicating that the activity of these channels can contribute to arrhythmia, atrioventricular dysfunction and SND exists. Global deletion of SK2 channels in mice is pro-arrhythmic and induces atrioventricular block (74; 153), thus suggesting that the alteration of SK current could lead to SND. Consistent with this hypothesis, it was shown that pharmacological inhibition of SK current recovers tachycardia-bradycardia syndrome in a model of Ca^{2+} overload caused by NCX1 deletion (32). Similarly, selective inhibition of the IK isoform SK4 was sufficient to reduce delayed afterdepolarizations and arrhythmic Ca^{2+} transients in a CPVT model (31). Mechanistically, hyperactivation of SK or IK channels in these models could lead to the hyperpolarization of SAN cells, as it has been shown in a model of neurons (154). Consistent with this hypothesis, selective inhibition of SK4 channels rescued SAN arrhythmia and SND, suggesting that the family of KCa channels could be an additional pharmacologic target to treat SND.

Human SAN as model of SND

The human SAN complex is a specialized and heterogeneous intramural 3D structure with multiple intranodal pacemakers and several specialized sinoatrial conduction pathways (SACPs), which are responsible for the transmission of electrical impulses to the right atrium (155; 156). The human SAN pacemaker-conduction complex relies heavily on a sophisticated machinery of multiple molecular pathways that communicate within the 3D structure in order to efficiently maintain physiologically relevant heart rate (**Figure 4**)(49; 66). However, there is a paucity of studies addressing mechanisms that contribute to automaticity and intranodal conduction directly in the human SAN complex at the molecular, cellular, and tissue levels (29).

Disease-induced remodeling of many of the molecular components critical to SAN function can lead to SND in humans (27; 156; 157). However, the majority of these molecular components critical to SAN function have been studied only in animal models, which may have significantly different functional and anatomical features compared to the human SAN, especially when studying aged and/or diseased human SAN with SND. The development of optimal treatments for SND will require in-depth knowledge of the mechanisms involved in robust human SAN rhythm regulation. However, substantial gaps exist in data on SAN functions obtained directly from the human SAN (157; 158), and considerable understanding is inferred from animal models, which may not reproduce the human clinical SND phenomena (156), or from clinical electrogram recordings that are restricted only to the atrial surface (155; 159).

Recent studies of the *ex vivo* human heart (49; 66; 160) directly address these limitations (**Figure 4** ***and*** **Supplementary Figure 4**). These studies of the *ex-vivo* human heart (49; 161) provide a unique opportunity to reinvent the translational study of human cardiac disease by applying state-of-the-art intramural mapping techniques consisting of near-infrared optical mapping (27; 29; 66; 161), 3D structural imaging (49; 160), and molecular mapping (59) to resolve mechanisms of human SAN function in normal and diseased hearts which are not possible *in-vivo*. To establish the efficient use of explanted human hearts with intact SANs, a rigorous and robust state-of-the-art approach has been developed to investigate e human hearts (with and without arrhythmia including SND) with 3D integrative approaches under *ex vivo* physiological conditions. Explanted human chronic failing hearts are acquired alive from the OSU cardiac transplantation program. Donor hearts with and without cardiac diseases and comorbidities (e.g. hypertension, diabetes and history of chronic smoke and alcohol consumption/abuse) are collected from LifeLine of Ohio, local organ donation organization (161).

These multidisciplinary integrated approaches have identified that SND may be orchestrated by (i) heterogeneous fibrotic structural remodeling in SACPs (49; 133; 160), (ii) compartment specific molecular remodeling in I_f pacemaker channels (HCN1/HCN2/HCN4)(59) and/or (iii) adenosine A1 receptor and I_{KAdo} (GIRK1/GIRK4) (**Supplementary Figure 4**) (27; 29; 161) and/or (iv) neuronal (n)Nav (nNav1.6) and cNav1.5 isoforms in human SAN (66). Recent, optical mapping studies revealed that unlike cNav, nNav may predominantly contribute to SAN intranodal conduction, rather than atrial conduction. On the other hand, cNav play important roles in both SAN pacemaking and conduction, especially during adenosine or atrial pacing challenges to prevent intranodal conduction failure. Impairment of Nav can lead to SAN exit block, disorganized intranodal pacemakers, and SAN micro- and macro-reentry. Furthermore, these functional observations are supported by higher expression of nNav (Nav1.1 and Nav1.6) and lower expression of cNav1.5 in human SAN pacemaker cells versus the surrounding atrial myocardium. Importantly, several nNav transcripts were vulnerable to cardiac remodeling associated with heart failure, cardiac hypertrophy and modifying risk factors including history of chronic alcohol consumption, which could promote a substrate for SAN arrhythmias (66).

From a clinical perspective, *ex-vivo* human SAN studies (66) highlight limiting the use of drugs that may block nNav channels especially when vagal tone is high, or in heart failure (162) and atrial fibrillation (163) patients with high plasma levels of adenosine. Furthermore, the *ex-vivo* human studies also suggest that region-specific SAN disease remodeling, such as fibrotic infiltration (133), significantly contribute to the intrinsic region-specific SAN conduction abnormalities, and arrhythmias (**Figure 4**). Disease-induced structural remodeling may exacerbate region-specific conduction abnormalities induced by both Nav blockers (66) and adenosine through GIRK channels and predispose to SND (**Supplementary Figure 4**). As such specific GIRK4 channel blockers (e.g. Tertiapin-Q) can prevent SAN pacemaker arrest and exit block induced by adenosine and parasympathetic hyperactivity and eventually be used in patients with atrial fibrillation and SND (29).

In summary, integrative *ex-vivo* human SAN studies revealed that the availability of multiple redundant structural compartments (intranodal pacemakers and SACPs) and molecular components in the human SAN are important backup mechanisms to robustly protect SAN conduction and pacemaking, and prevent rhythm failure and SND, in the context of multiple disease-induced conduction impairments (29; 133; 160).

Conclusions and perspectives

The development of new pharmacologic and molecular strategies to handle chronic primary and secondary forms of SND has been complicated by a lack of knowledge about the mechanisms underlying this complex pathology. However, new and unexpected mechanisms of SND have been described during the last years using human genetics, animal models of SND, as well as *ex vivo* SAN from human hearts with history of secondary SND. These investigations have indicated potentially innovative pharmacologic targets to manage SND such as GIRK and SK channels. Furthermore, exploring animal models of SND and human SAN may help redirect the application of antiarrhythmic drugs and create innovative therapies for concomitant control of SND and associated arrhythmias. We expect that additional new SND mechanisms will be unraveled in the coming years with extensive functional genomics exploration of primary familial SND and development of new cellular and animal models of secondary forms of SND.

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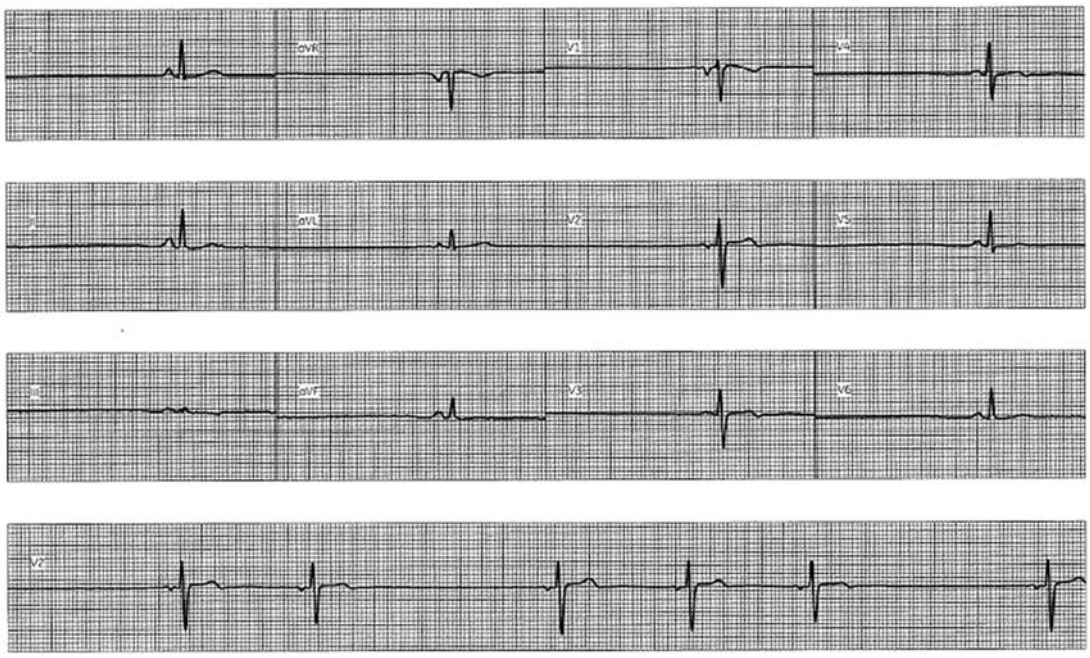

Figure 1. 12 lead ECG showing severe sinus bradycardia and rhythm strip showing sinus pauses as a manifestation of SND. Baseline heart rate was 45 bpm. Courtesy of Dr. Francis Marchlinski, University of Pennsylvania.

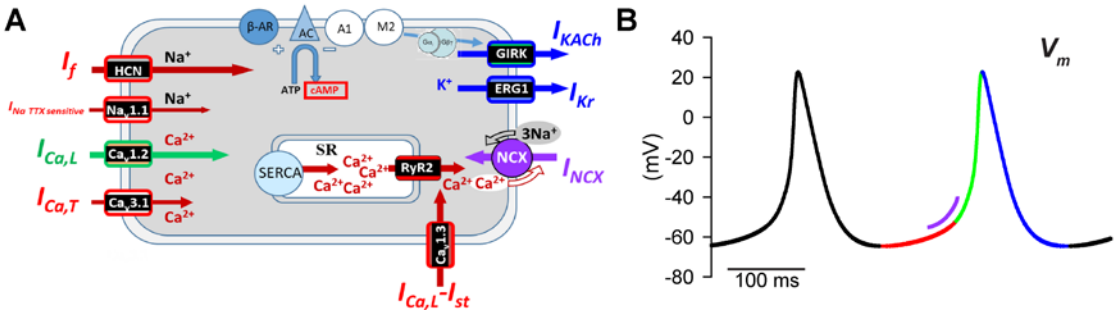

Figure 2. (A) Schematic of a sinoatrial node (SAN) pacemaker cell showing the main membrane bound receptors and ion channels together with RyR2, the SR calcium pump SERCA and the Na^+/Ca^{2+} exchanger (NCX1). (B) Simulated transmembrane potential (V_m) during a spontaneous action potential (AP) generated by a computational model of the mouse SAN myocyte (164), with color-code corresponding to the major AP phases [colors also indicate corresponding underlying currents in (A)]: Diastolic depolarization (*red*), AP upstroke (*green*), repolarization (*blue*). See also the extended version of this Figure in the online Appendix (Supplementary Figure 2).

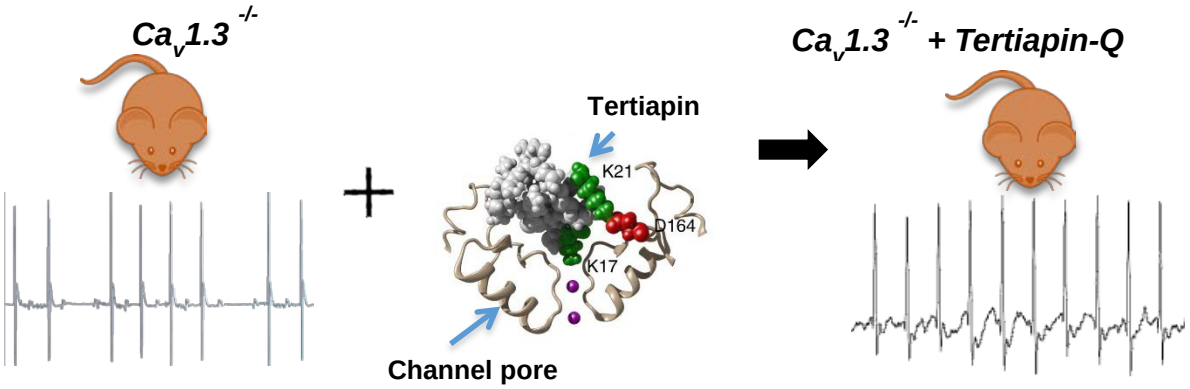

Figure 3. Rescuing of SND and atrioventricular dysfunction by I_{KACh} targeting. Tertiapine-Q rescues SND in *$Ca_V1.3^{-/-}$* mice. Administration of the GIRK pore blocker tertiapin-Q (tertiapin, central panel) normalizes heart rate (right panel). The central panel shows a close up view of a structural model of tertiapin bound to the GIRK pore. K17 and D164 indicate residues important for peptide activity. Adapted from reference (35), with permission by Whiley and Son.

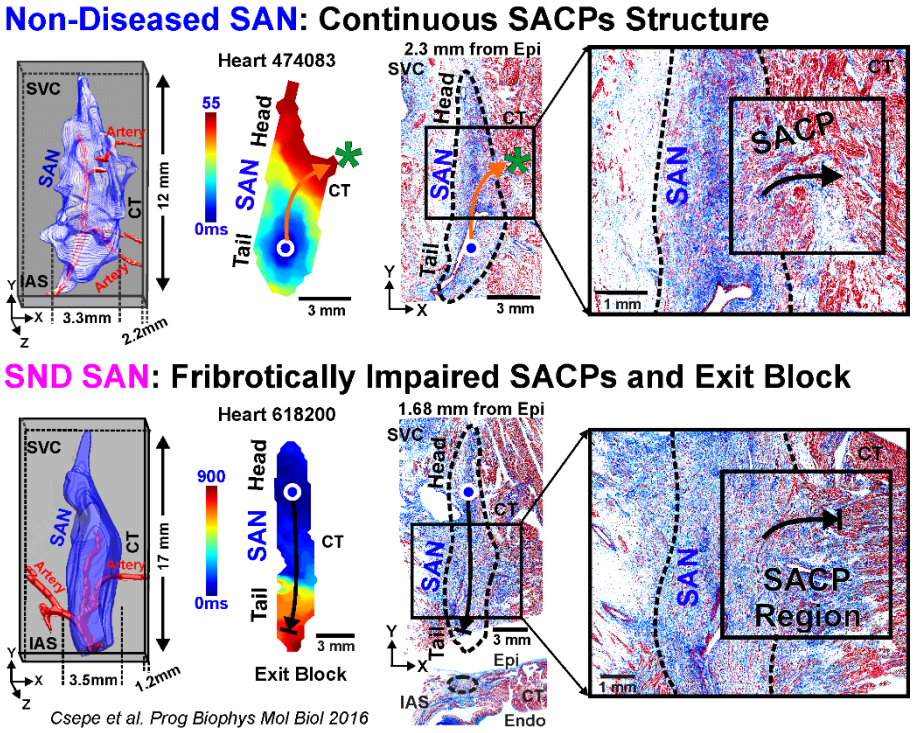

Figure 4. Functional and structural imaging of human SAN with history of SND

(Left to Right) 3D reconstruction of human SAN; SAN activation exciting atria (top, non-diseased) or exit block (bottom, SND); histology staining of SAN complex; magnified section of lateral middle SACP showing continuously coupled myocytes (top) or fibrotic disruptions (bottom). Epi-epicardium; Endo-endocardium. Region of intranodal conduction block coincided with strands of intranodal fibrosis and the SAN artery. CT-crista terminalis; IAS-interatrial septum; SAN-sinoatrial node; SANCP –SAN Conduction pathway; SVC-superior vena cava. *Adapted From Csepe et al., 2016. Progress in biophysics and molecular biology 120 (1-3), 164-178. (49)*