REVIEW

The Anatomy and Physiology of the Sinoatrial Node—A Contemporary Review

OLIVER MONFREDI, M.B.CH.B.,* HALINA DOBRZYNSKI, PH.D.,* TAPAS MONDAL, M.D.,† MARK R. BOYETT, PH.D.,* and GWILYM M. MORRIS, BM.B.CH.*

From the *Cardiovascular Medicine, Faculty of Medical and Human Sciences, University of Manchester, Core Technology Facility, Manchester, United Kingdom; and †McMaster Children's Hospital, Hamilton, Ontario, Canada

The sinoatrial node is the primary pacemaker of the heart. Nodal dysfunction with aging, heart failure, atrial fibrillation, and even endurance athletic training can lead to a wide variety of pathological clinical syndromes. Recent work utilizing molecular markers to map the extent of the node, along with the delineation of a novel paranodal area intermediate in characteristics between the node and the surrounding atrial muscle, has shown that pacemaker tissue is more widely spread in the right atrium than previously appreciated. This can explain the phenomenon of a "wandering pacemaker" and concomitant changes in the P-wave morphology. Extensive knowledge now exists regarding the molecular architecture of the node (in particular, the expression of ion channels) and how this relates to pacemaking. This review is an up-to-date summary of the current state of our appreciation of the above topics. (PACE 2010; 33:1392–1406)

sinoatrial node*, *electrophysiology*, *pacemaking*, *ion channels

Introduction

The sinoatrial node (SAN) of healthy humans is the primary pacemaker of the heart. It is usually depicted in textbooks as a very small discrete area near where the superior vena cava enters the right atrium. This is an oversimplification, and consequently many physicians are unaware of the extent and complexity of the SAN. The SAN is an anatomically and electrophysiologically heterogeneous structure that expresses a unique set of ion channels necessary for the generation and propagation of the action potential. Dysfunction of the ion channels by remodeling in disease states or as a result of inherited mutations can lead to impaired SAN function.

The Discovery of the Sinoatrial Node

The SAN was discovered over 100 years ago in 1907 by Sir Arthur Keith, who examined human hearts histologically with the initial intention to define the mechanisms behind closure of the great veins during atrial systole.[1,2] During these examinations he defined "a small condensed area of tissue, just where the cava sank into the auricle," but did not believe it to be functionally important (Fig. 1A).[1] Following Tawara's discovery and description of the bundle of His while working in the laboratory of Aschoff,[3] Keith's work received new impetus and (working with medical student Martin Flack) he was inspired to study the hearts of smaller mammals including moles.[1] Here, they discovered "a wonderful structure in the right auricle," which was reproducibly present in all the subsequent hearts studied.[2] It was not until 4 years later that this was discovered to be the point of initial cardiac stimulation.[4,5]

Basic Anatomy

The crescent-shaped human SAN lies at the junction of the superior vena cava and the right atrium. The myocytes comprising the SAN are small, pale, with poorly developed sarcomeres and sarcoplasmic reticulum.[6–8] They are set in an irregularly bordered highly fibrous connective tissue matrix, allowing discrimination from the surrounding non-nodal tissue, which contains much less connective tissue on basic histological examination (Fig. 1B).[9] The SAN is signposted by the presence of a relatively large artery and it occupies a subepicardial position next to the crista terminalis (Fig. 1A and B).[9] Early depictions of the node from the 1960s demonstrated it to be a relatively limited structure in the crest of the right atrial appendage, at the junction of the superior vena cava and right atrium.[10,11] Later reconstructions showed the node

Work was supported by grants from the British Heart Foundation, Medtronic Inc., British Cardiovascular Society, Bristol-Myers-Squibb, and BBSRC.

Address for reprints: M.R. Boyett, Ph.D., Cardiovascular Medicine, Faculty of Medical and Human Sciences, University of Manchester, Core Technology Facility, 46 Grafton Street, Manchester M13 9NT, United Kingdom. Fax: 44-161-275-1183; e-mail: mark.boyett@manchester.ac.uk

Received January 25, 2010; revised March 21, 2010; accepted May 21, 2010.

doi: 10.1111/j.1540-8159.2010.02838.x

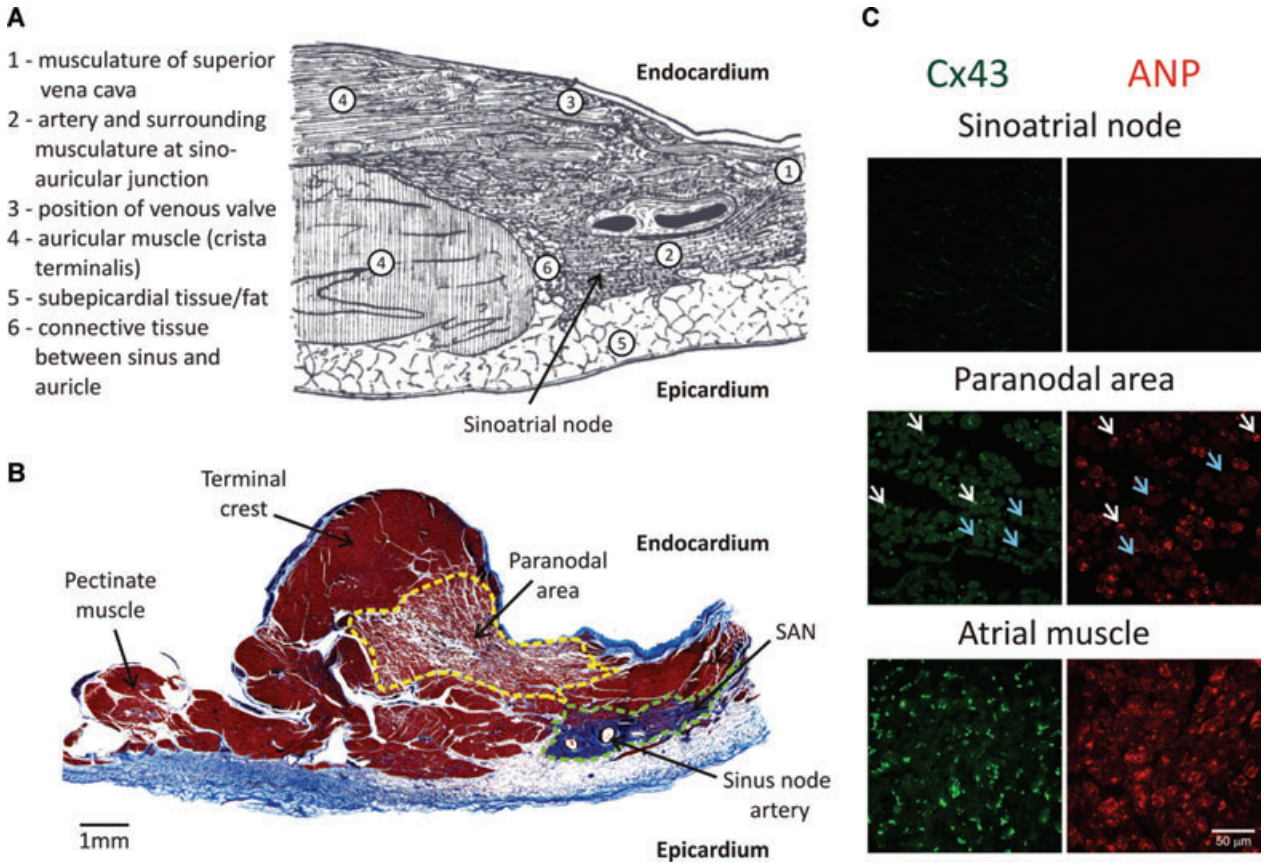

Figure 1. *(A) The "sino-auricular junction in human heart" from the original description of the SAN from Keith and Flack published in 1907.[2] A drawing of a tissue section through the terminal crest and SAN is shown. (B) Tissue section through the terminal crest and SAN of the human heart stained with Masson's trichrome (dark-blue/black = nuclei, pink = myocytes, royal blue = connective tissue). The SAN and paranodal area are outlined with dashed lines. From the study of Chandler et al.[16] published in 2009. (C) Immunolabeling of Cx43 (green spots) and ANP (red spots) in the SAN, paranodal area, and atrial muscle in the human. Cx43 and ANP are absent from the SAN, present in some cells in the paranodal area, and present in all myocytes in the atrial muscle. From Chandler et al.[16]*

as a more diffuse, elaborate structure, usually extending down the inferolateral aspect of the crista terminalis in a cigar shape.[9] There are various indications that the SAN (or at least the pacemaker tissue) is an extensive structure:

1. In the rabbit, the SAN extends down the crista terminalis toward the entrance to the inferior vena cava (Fig. 2).[9] Variation is commonplace and alternative arrangements include extension of the SAN across the crest of the right atrial appendage to sit in the interatrial groove.[12]

2. The embryonic development of the SAN is controlled by a T-box transcription factor, Tbx3.[13] Tbx3 is only expressed in the cardiac conduction system of the heart, i.e., the SAN, the atrioventricular node (AVN), and the first parts of the bundle branches.[14] It acts as a transcriptional regulator that induces and maintains formation of the SAN, while inhibiting expression of atrial genes. In the embryonic mouse heart, its presence has given valuable clues to the true extent of the SAN—nodal tissue, defined by the presence of Tbx3, continues from the superior vena cava down the crista terminalis to the inferior vena cava and ultimately to the AVN.[15]

3. Recent work has shown an extensive "paranodal area" in humans, located within the crista terminalis close to, but not continuous with, the SAN (Fig. 1B).[16] It is likely to be more extensive than the SAN (see below). The paranodal area comprises a mixture of loosely packed nodal and atrial myocytes and has a molecular architecture in some respects distinct and intermediate to the SAN and atrial muscle.[16] Whereas in the SAN there is no Cx43 (connexin43—responsible for electrical coupling between cardiac myocytes) and no atrial natriuretic peptide (ANP), and in the

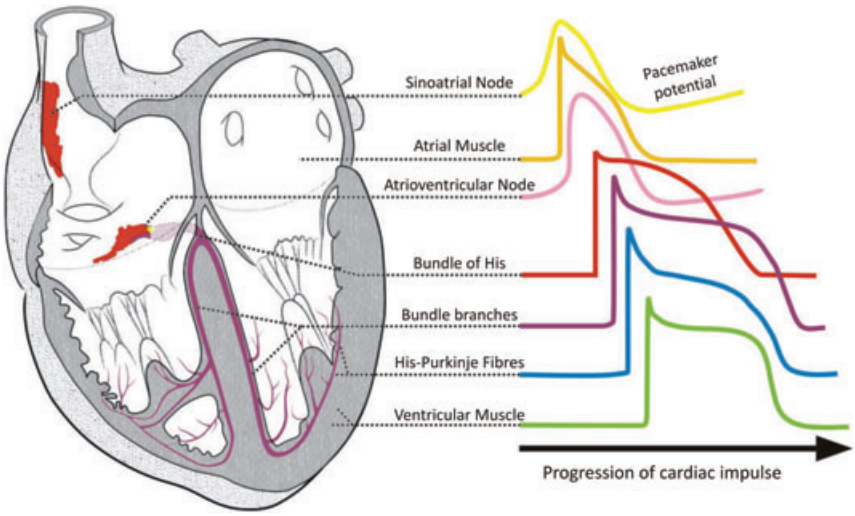

Figure 2. *Schematic diagram of the rabbit heart, showing the proposed position of the components of the cardiac conduction axis. The SAN and AVN, shown in red, are based on accurate computer reconstructions.*[52,121] *The His-Purkinje network, shown in purple, is schematic. A schematic diagram of typical action potential morphologies in the different regions of the heart is shown on the right.*

atrial muscle there is both, in the paranodal area there is a heterogeneous mixture of myocytes, some of which express Cx43 and ANP while others do not (Fig. 1C).[16] Ion channels are ultimately responsible for the electrical activity of a tissue and, as expected, the expression of ion channels in the SAN is different from that in the working myocardium.[16] In certain important cases, the expression of ion channels in the paranodal area is intermediate between that of the SAN and atrial muscle, but in other cases it is distinct from that in both the SAN and atrial muscle.[16] At present, the role of the paranodal area can only be speculated on: it may facilitate the exit of the action potential from the SAN into the atrial muscle.[16] Alternatively, it may be involved in normal pacemaking—perhaps the paranodal area is acting as the leading pacemaker site in the patient in Figure 3B. The paranodal area could also be responsible for conduction discrepancies that favor the genesis of cristal tachycardias (atrial tachycardias arising along the length of the crista terminalis).[17] However, it is worth mentioning that no functional studies have thus far been performed on the paranodal area, and hence the functional significance of the above anatomical findings remains unclear.

4. There are multiple features of the human SAN that make it difficult to ablate or modify with endocardial catheter techniques (as may be required in patients with inappropriate sinus tachycardia), including the caudal proximity of the thick crista terminalis and the cooling effects of the nodal artery.[9] However, a major reason that the SAN is difficult to ablate is its extensive location[18–20]—in the dog Kalman et al.[21] had to ablate the entire crista terminalis from the superior to the inferior vena cava to stop sinus rhythm (a distance of 3–4 cm in this species). Similar problems have been experienced when trying to ablate the human SAN.[20] This is clearly inconsistent with the textbook picture of the SAN, but it is consistent with the other indications above of the extensive nature of the SAN.

The exact mechanism of how the electrical impulse exits from the SAN is far from clear. An area of functional medial block (i.e., toward the interatrial septum) has long been recognized to occur within the rabbit SAN.[22] Early work by Bromberg et al.[23] demonstrated that in the dog, mapping of extracellular potentials from the SAN and right atrium showed exit sites located at the cranial and caudal ends of the node, and that ablation of these discrete sites lead to SAN exit block. Schuessler[24] subsequently put forward a model of the sinus node whereby it was not diffusely anatomically continuous with the atrial myocardium, rather it was attached to the atrial musculature only at a limited number of discrete exit sites. The recent work of Fedorov et al.[25] went further, suggesting that the dog SAN (with its similar three-dimensional arrangement to that of the human) is anatomically surrounded by a loop of both vessels and connective tissue that

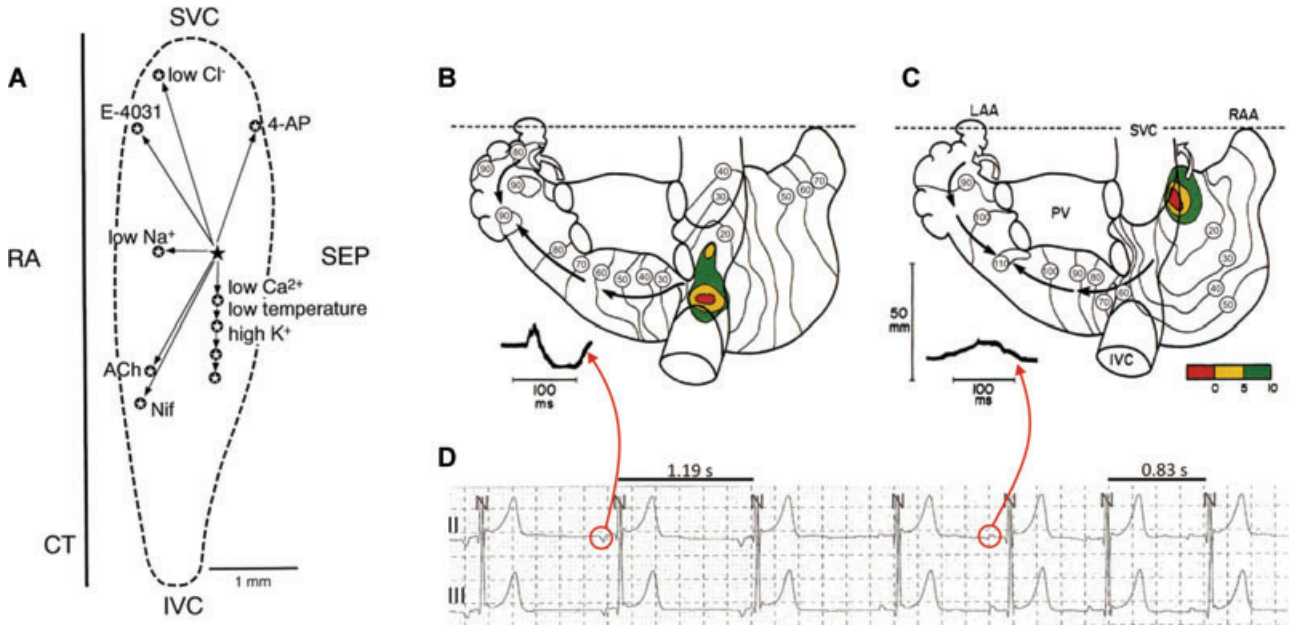

Figure 3. *The position of the leading pacemaker site is highly variable. (A) Pacemaker shift in the rabbit SAN. The position of the leading pacemaker site under basal conditions is shown by the black star and under the indicated conditions by the white stars. From Boyett et al.*[22] *(B, C) The position of the leading pacemaker site in two patients during cardiac surgery. Drawings of the atria (dorsal view) are shown. The activation sequence of the atria is shown by the isochrones in milliseconds and the associated P wave is shown in the inset. The leading pacemaker site is highlighted in red. In the first patient (B), the leading pacemaker was at an inferior site near the inferior vena cava giving an upward vector of atrial activation and a negative P wave in lead aVF. In the second patient (C), the leading pacemaker was near the superior vena cava and the vector of atrial activation was inferior and the P wave in lead aVF was positive. From Boineau et al.*[122] *(D) Spontaneous pacemaker shift in the human. The trace shows inferior leads II and III during Holter monitoring of a healthy 17-year-old female. Note that a negative P wave was associated with a long R-R interval and a positive P wave was associated with a shorter R-R interval. This presumably reflects a change in the position of the leading pacemaker site from an inferior to a superior site.*[123] *Abbreviations: 4-AP, 4-aminopyridne (blocker of the transient outward K^+ current); ACh, acetylcholine; CT, crista terminalis; E-4031, blocker of the rapid delayed rectifier K^+ current; IVC, inferior vena cava; LAA, left atrial appendage; Nif, nifedipine; PV, pulmonary veins; RA, right atrium; RAA, right atrial appendage; SEP interatrial septum; SVC, superior vena cava.*

lead to anatomical and physiological conduction block on both sides of the node (medially toward the interatrial septum and laterally toward the crista terminalis). They demonstrated (with optical mapping of action potentials) that SAN exit pathways predominantly exist superiorly and inferiorly and are few in number, while action potentials that initially travel in the transverse direction propagate markedly more slowly than those that travel in the supero-inferior direction, and eventually disappear without ever leaving the node. Such an arrangement would have certain physiological advantages (good electrical insulation from the surrounding hyperpolarizing influences of the atrial muscle), and some disadvantages (damage to the exit pathways can easily lead to SAN exit block, bradycardia, syncope, and sudden cardiac death). However, the detailed work of Sanchez-Quintana et al.[9] completely failed to demonstrate any evidence for an insulating sheath of fibrous tissue surrounding the SAN on any side in the 47 adult human hearts studied. They also demonstrated multiple radiations of nodal tissue interdigitating with normal atrial myocardium, leading them to suggest that there was potential for multiple breakthroughs of the nodal wavefront. Likewise, Matsuyama et al.[26] did not demonstrate any histological or anatomical areas of likely conduction blockade in their thorough study of the posterolateral right atrium of human samples taken at post-mortem—see Figure 4. They demonstrated that at the various levels studied within the SAN, there appeared to be continuity between SAN and atrial muscle cells. It may be, therefore, that the presence of discrete exit sites is a functional rather than an anatomical phenomenon. Further work is undoubtedly required to elucidate the exact

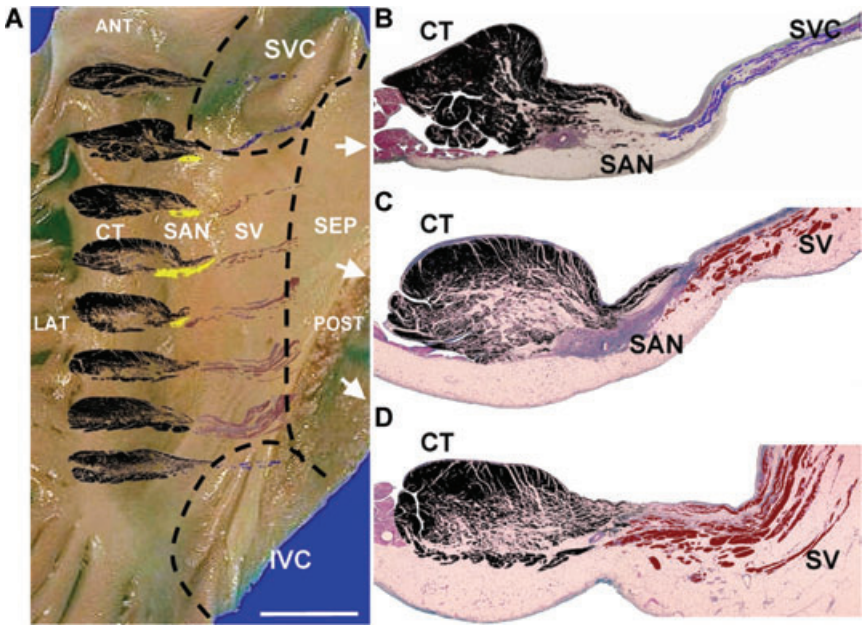

Figure 4. *Extent of the human SAN. (A) Photograph of a human SAN preparation on which are superimposed tissue sections (cut perpendicular to the crista terminalis through the SAN) at the levels shown. Black, atrial muscle (and paranodal area); yellow, SAN; blue, superior vena cava; violet, sinus venosus. (B–D) Further tissue sections cut at the levels shown by the white arrows. Black, atrial muscle (and paranodal area); gray, SAN; blue, superior vena cava; red, sinus venosus. ANT, anterior; CT, crista terminalis; IVC, inferior vena cava; LAT, lateral; POST, posterior; SEP, septum; SVC, superior vena cava; SV, sinus venosus. Azan-Mallory stain. Bar = 10 mm. From Matsuyama et al.*[26]

mechanism of exit of action potentials from the SAN.

Physiology

Pacemaking in the intact SAN is not limited to a single anatomical area. Figure 3A demonstrates that the leading pacemaker site (the site of first activation) in the rabbit moves in response to physiological stimuli, a phenomenon known as "pacemaker shift" or the "wandering pacemaker." Figure 3B and C demonstrates variation in the site of the leading pacemaker, this time in the human (data obtained by activation mapping during cardiac surgery). In one patient, the leading pacemaker site occurred at the junction of the superior vena cava with the right atrium, by convention the location of the SAN (Fig. 3C). However, in the second patient, the leading pacemaker site occurred at the junction of the inferior vena cava with the right atrium (Fig. 3B). By convention, the SAN is not thought to extend to the inferior vena cava in the human (yellow area in Fig. 4A). In the example shown in Figure 3B, therefore, the leading pacemaker site is outside the anatomical SAN. However, as mentioned above, in the embryonic mouse heart Tbx3-expressing nodal tissue extends from the superior to the inferior vena cava. Furthermore, the paranodal area may extend down to the inferior vena cava—Figure 4 shows a diffuse extension of myocytes as far as the inferior vena cava that may represent the paranodal area. The pacemaker shift is likely to be responsible for variations in P-wave morphology often seen, but ignored. Figure 3B and C shows that the shift of the leading pacemaker site resulted in a change in the polarity of the P wave. Figure 3D shows an electrocardiogram (ECG) recorded from a 17-year-old girl who attended McMaster Children's Hospital (Canada) for investigation of palpitations. Her 12-lead ECG, 24-hour ambulatory ECG, and echocardiogram were essentially normal. However, during sleep there was a spontaneous change in the polarity of the P-wave vector in the inferior ECG leads from negative to positive (accompanied by an increase in the heart rate; Figure 3D), presumably due to a superior shift of the leading pacemaker site. In an *ad hoc* screen of ECG recordings from ~300 human subjects at a private clinic in Manchester (UK), ~1% of subjects had a negative P wave in an inferior lead. The phenomenon of pacemaker shift demonstrates that the SAN is heterogeneous

rather than uniform. In the dog, it has been noted that sympathetic nerve stimulation quickens the heart rate and causes a superior shift of the leading pacemaker, whereas vagal nerve stimulation slows the heart rate and causes an inferior shift of the leading pacemaker.[27] This has resulted in the concept of a hierarchy of pacemakers within the SAN, with the slowest located inferiorly and the fastest superiorly.[27] An alternative, though equally viable, explanation of pacemaker shift and difference in P-wave morphology is that there is differential exit of the action potential from the SAN. For example, it may exit at a superior site in Figure 3C, and an inferior site in Figure 3B.

Action potential morphology differs throughout the heart, the action potential of the SAN differing markedly from the action potential of the working myocardium (Fig. 2). It has been known since the 1940s that the hallmark of spontaneously active cardiac tissue is diastolic depolarization (also termed the "pacemaker potential"),[28] which allows the triggering of an action potential when a threshold potential is reached (Fig. 5). Furthermore, the SAN has a relatively depolarized (less negative) membrane potential during diastole with a slower upstroke and smaller overshoot (Fig. 5).[29]

Concepts in Pacemaking

During the pacemaker potential, the SAN myocyte is depolarized (i.e., the membrane potential becomes more positive; Figure 5). Inward ionic current (i.e., flow of positively charged cations across the cell membrane via specific ion channels into the myocyte) causes depolarization (i.e., a positive shift in the membrane potential), while outward ionic current (i.e., flow of positively charged cations out of the myocyte via other ion channels) causes repolarization (i.e., a negative shift in the membrane potential). The contribution of different ionic currents (each flowing through a unique ion channel) to the pacemaker potential has been revealed by recording of the ionic currents using the voltage clamp technique and specific pharmacological blockade of the ionic currents, and the role of individual ion channels by transgenic knockout or mutant animals. The pacemaker potential (diastolic depolarization) is the result of the absence of one outward current, the decay of another outward current, and an increase in various inward currents as shown in Figs. 5 and 6 and discussed below.

K^+ Currents

The working myocardium has a stable resting potential (Fig. 5) and this is generated by an outward current, the confusingly named inward rectifier K^+ current, $I_{K,1}$, carried by $K_{ir}2$ channels, such as $K_{ir}2.1$. The SAN has no stable resting potential, because it lacks $I_{K,1}$ and $K_{ir}2.1$,[16] and this sets the scene for pacemaking (in the ventricles, simply knocking out $K_{ir}2.1$ allows pacemaking to occur[30]). During the action potential, the delayed rectifier K^+ current, I_K, is activated and is responsible for repolarization of the myocyte at the end of the action potential. After the action potential, I_K decays and this allows other inward currents (see below) to depolarize the myocyte—in this way, the decay of I_K is believed to be

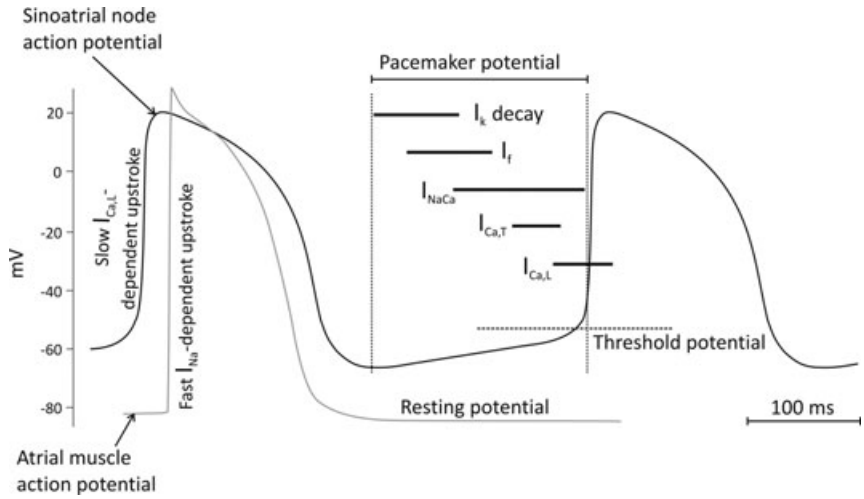

Figure 5. *Typical action potentials recorded from atrial muscle (light gray) and the center of the SAN (black). The temporal contributions of the main ionic currents to the pacemaker potential are shown by the black bars. Adapted from Boyett.*[115]

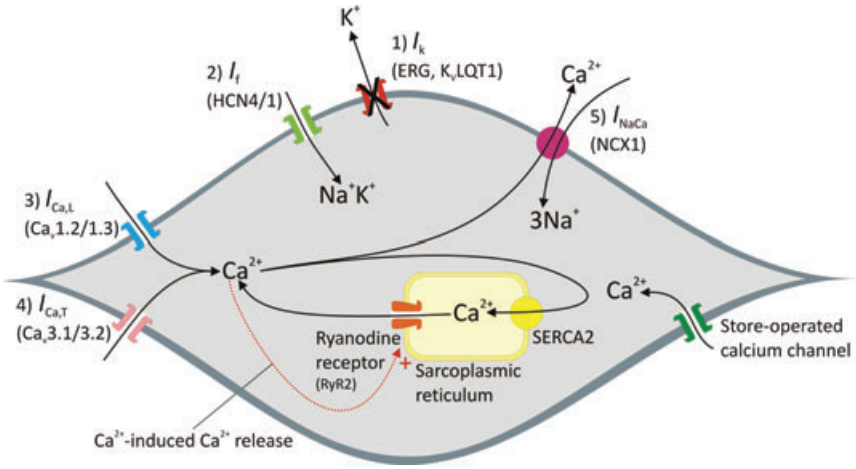

Figure 6. *Five ionic currents involved in SAN pacemaking. A SAN myocyte is shown. Membrane clock: during the pacemaker potential, there is a voltage-dependent decay of outward current (I_K or outward rectifier K^+ current; 1) and a voltage-dependent activation of at least three inward currents, I_f (funny current; 2), $I_{Ca,L}$ (L-type Ca^{2+} current; 3) and $I_{Ca,T}$ (T-type Ca^{2+} current; 4). Ca^{2+} clock: during the final phase of the pacemaker potential, there is an activation of inward I_{NaCa} (Na^+-Ca^{2+} exchange current; 5) in response to a spontaneous release of Ca^{2+} from the sarcoplasmic reticulum via the ryanodine receptor (RyR2). Because the Na^+-Ca^{2+} exchanger exchanges one intracellular Ca^{2+} ion for three extracellular Na^+ ions, it is electrogenic and generates an inward current (I_{NaCa}) on removing Ca^{2+} from the cell. Ca^{2+} release from the sarcoplasmic reticulum also occurs as a result of Ca^{2+}-induced Ca^{2+} release in response to Ca^{2+} entry into the cell via $I_{Ca,L}$ and $I_{Ca,T}$. The sarcoplasmic reticulum is replenished with Ca^{2+} by reuptake of Ca^{2+} into the sarcoplasmic reticulum via SERCA2 (Sarco/Endoplasmic Reticulum Ca^{2+} ATPase). Store-operated Ca^{2+} channels (SOCC) at the cell surface membrane may also help to replenish the sarcoplasmic reticulum with Ca^{2+}.*

responsible for the earliest part of the pacemaker potential (Figs. 5 and 6).[31] I_K has fast and slow components, $I_{K,r}$ and $I_{K,s}$, carried by the K^+ channels, ERG and K_vLQT1, respectively.

Funny Current

Perhaps the best-known ionic current in the SAN is the "funny current" (I_f), an inward current carried by Na^+ and K^+ ions, which is specifically activated at hyperpolarized membrane potentials.[32,33] The ion channels responsible for I_f are the hyperpolarization-activated cyclic-nucleotide gated gene family (HCN), of which there are four isoforms.[34] In humans, the predominant cardiac isoforms are HCN1 and HCN4.[16] The characteristics of I_f are intermediate between those of HCN1 and HCN4, suggesting that the biologically active channels are heteromultimers of HCN1 and HCN4 subunits.[35] The importance of I_f in pacemaking is suggested by the abundance of HCN1 and HCN4 in the SAN and their absence in the atrial muscle.[16] Furthermore, forms of congenital idiopathic sinus bradycardia have been identified, caused by heterozygous dominant-negative mutations in HCN4 (see below).[36–38] In the rabbit, block of I_f by CsCl leads to a slower pacemaker potential and, therefore, a prolongation (of around 14%) of the period between successive action potentials.[39] In the isolated rabbit SAN preparation, block of I_f by the specific heart rate lowering drug, ivabradine, slows the heart rate by 24% (when at a concentration of 3 μM). When given to humans, ivabradine at a dose of 5 mg twice daily causes a mean decrease in resting heart rate of 9.5 beats per minute.[40] Both of these effects of ivabradine occur mainly by decreasing the slope of the pacemaker potential.[41] Sympathetic nerve stimulation quickens the pacemaker potential and leads to an increased pacing rate.[42] HCN channels are suggested to be central to this process, because cAMP produced in response to β1-adrenoceptor activation following sympathetic nerve stimulation modulates the electrophysiological properties of the HCN channels via a cytoplasmic cyclic nucleotide binding domain.[43] The central role of I_f in pacemaking is controversial—for example, a knockout mouse expressing no

HCN4 and reduced I_f displays sinus pauses, but no bradycardia or complete lack of pacing as predicted by some investigators.[44] However, Stieber et al.[45] demonstrated that mice deficient in HCN4 die *in utero*, having hearts that show slowed cardiac conduction and an absence in primitive pacemaking cells at post-mortem examination. This, they suggest, shows that HCN4 is essential for the generation of pacemaker potentials in the heart. Recently, we have shown that block of I_f by Cs^+ increases heart rate variability (O. Monfredi, unpublished data) and this suggests that an important role of I_f is to stabilize the heart rate as suggested by Noble et al.[46]

Ca^{2+} Currents

Ca^{2+} channels are activated by the rising membrane potential late during the pacemaker potential (Fig. 5). The L-type Ca^{2+} current ($I_{Ca,L}$) in the SAN is dependent on the Ca^{2+} channel, $Ca_v1.3$ (and, perhaps in addition, $Ca_v1.2$), while in the working myocardium it is exclusively $Ca_v1.2$ that carries this current.[47] $Ca_v1.3$ has a more negative threshold (activation) potential than $Ca_v1.2$; thus, it is a more appropriate channel for pacemaking tissues because it is activated earlier in the pacemaker potential.[48] Block of $I_{Ca,L}$ with nifedipine abolishes pacemaking in central SAN myocytes, because this current is responsible for the slow action potential upstroke in the SAN (the Na^+ channel, $Na_v1.5$, responsible for the fast action potential upstroke in the working myocardium, is mostly absent—see below).[47,49] Why nifedipine should have no effect on heart rate in humans therefore is intriguing. One possibility is that although nifedipine abolishes pacemaking in central $I_{Ca,L}$-dependent SAN tissue, it accelerates pacemaking in peripheral I_{Na}-dependent SAN tissue,[49] and thus its overall effect on rate in humans is balanced out (see Fig. 3A, which demonstrates a shift in leading pacemaker site in response to nifedipine, from central to peripheral, negating any negatively chronotropic effects that might be otherwise expected).

Transgenic mice lacking $Ca_v1.3$ are bradycardic with sinus dysrhythmia.[48,50] The T-type Ca^{2+} current ($I_{Ca,T}$) is dependent on the Ca^{2+} channels, $Ca_v3.1$ and $Ca_v3.2$, and has been found in all cardiac myocytes displaying automaticity, including SAN myocytes.[51] These channels are significantly more abundant in the SAN than the working myocardium.[16] Block of $I_{Ca,T}$ with Ni^{2+} prolongs the cycle length by 14% and knockout of the $Ca_v3.1$ gene in the mouse leads to bradycardia and increased SAN recovery time.[48,51]

Na^+ Current

The inward Na^+ current (I_{Na}) is important in the working myocardium and is carried by the $Na_v1.5$ channel (encoded by the SCN5A gene). It is responsible for the fast upstroke of the action potential in the working myocardium. This current is abundant in the working myocardium and in the periphery of the SAN, but is absent from the center of the SAN, explaining the slower upstroke of the action potential seen here (Fig. 5).[52–54] Despite documented absence of $Na_v1.5$ from the center of the SAN,[16] $Na_v1.5$ knockout mice still show bradycardia, abnormally long SAN conduction times, and frequent SAN conduction block.[55–60] Likewise, mutations in the gene for $Na_v1.5$ (SCN5A) in humans have been associated with familial sick sinus syndrome. These effects are surprising given the lack of $Na_v1.5$ in the center of the node, but are postulated to arise from impaired channel function at the periphery of the SAN.[52] Human SAN myocytes excised from a patient with inappropriate sinus tachycardia showed a large inward current resembling I_{Na}.[61] These myocytes might represent transitional (peripheral) myocytes rather than true central SAN myocytes, but this finding might also suggest that I_{Na} may be more important in the human SAN than traditionally thought.

"Ca^{2+} Clock"

So far, the "membrane clock" underlying pacemaking has been described, i.e., the time- and voltage-dependent decay of I_K and time- and voltage-dependent activation of I_f, $I_{Ca,L}$, $I_{Ca,T}$, and I_{Na}. In addition, there is a "Ca^{2+} clock" (Fig. 6).[62] Within cardiac myocytes, the sarcoplasmic reticulum acts as an intracellular Ca^{2+} store. In the SAN, late during the pacemaker potential, Lakatta and colleagues[63] have shown that Ca^{2+} is spontaneously released from the sarcoplasmic reticulum into the cytoplasm via the ryanodine receptor, RYR2 (actually a Ca^{2+} channel). The intracellular Ca^{2+} is then extruded from the myocyte via the Na^+-Ca^{2+} exchanger (NCX1; Fig. 6). This then generates a significant inward current (I_{NaCa}), because the exchanger is electrogenic—it exchanges three Na^+ ions for each Ca^{2+} ion—this has been suggested to be responsible for the final exponential phase of the pacemaker potential (Fig. 5). The role of the Ca^{2+} clock in pacemaking is controversial.[52] For example, block of sarcoplasmic reticulum Ca^{2+} release by ryanodine has been reported to reduce the pacemaker rate by as little as −5 to +27%,[64] but in other studies by as much as +100%.[63] However, block of I_{NaCa} by Li^+ abolishes pacemaking in

SAN myocytes.[63] Store-operated Ca^{2+} channels at the cell surface membrane may be responsible for regulating the amount of Ca^{2+} within the sarcoplasmic reticulum (Fig. 6). Block of these channels reduces the pacemaking rate by 25%.[65] The mechanism is not entirely clear, but it may be that the repletion of intracellular Ca^{2+} stores by the store-operated Ca^{2+} channels sets the frequency of the Ca^{2+} clock.

Gap Junctions and Electrical Coupling

Gap junctions (nonspecific ion channels connecting neighboring myocytes) are responsible for the electrical coupling between cardiac myocytes and the propagation of the action potential throughout the heart. Gap junctions are comprised of connexins (Cx). Cx43 is abundantly expressed in the working myocardium, where it forms relatively large conductance gap junctions. Cx43 is responsible for the electrical coupling between working myocytes and the high conduction velocity of the action potential in the working myocardium. In contrast, Cx43 is not expressed in the center of the SAN.[66] Instead Cx45 is expressed in the SAN, where it forms small conductance gap junctions and, consequently, the myocytes in the center of the SAN are poorly electrically coupled.[66] As a result, the conduction velocity of the action potential in the center of the SAN is slow, but more importantly the center of the SAN is electrically insulated from the surrounding atrial muscle.[66] This is important, because the atrial muscle (which does not show pacemaker activity) can suppress the pacemaker activity of the SAN.[66] Toward the periphery of the SAN, the electrical coupling improves, with expression of Cx43 and 45 demonstrated in the periphery of the rabbit SAN.[66] Furthermore, interdigitations between SAN and atrial myocytes are seen in the periphery, theoretically facilitating the propagation of the action potential from the SAN into the surrounding atrial muscle.[66] Gap junction dysfunction significantly impacts on normal pacemaking. Cx40 forms large conductance gap junction channels. As in the case of Cx43, Cx40 is present in the atrial muscle, but largely absent from the SAN.[16] Nevertheless, Cx40 knockout mice demonstrate bradycardia with SAN exit and entry block, as well as a prolongation of SAN conduction time.[67]

It is important to electrically insulate the SAN from the surrounding atrial muscle as discussed above, and this is probably why the area of contact between the SAN and atrial muscle is restricted, and the SAN is surrounded by fatty tissue (Figs. 2 and 4).

Sinoatrial Node Remodeling and Sinoatrial Node Dysfunction

SAN dysfunction as a clinical entity includes a variety of disorders, including inappropriate sinus bradycardia, sinus arrest, chronic atrial fibrillation, and tachycardia-bradycardia syndrome. It is a common problem in clinical cardiology, and one of the commonest indications for insertion of permanent pacing systems.[68] Its frequency is expected to increase significantly as the general population continues to live longer. Its etiology includes structural abnormalities of the node, drug effects, and pathological autonomic influences. Rather than a single entity, SAN dysfunction is better conceptualized as a spectrum of disorders, whereby a number of different pathophysiological mechanisms lead to a very similar disease phenotype (Fig. 7):

1. **Familial SAN dysfunction**—As detailed below, SAN dysfunction is significantly more common in elderly patients. However, it is known to occur in the fetus, infants, children, and young adults,[69–71] and a large number of these "early onset" cases are associated with direct SAN injury from previous cardiac surgery for congenital heart defects. However, a significant number of people who experience SAN dysfunction in the first few decades of life have no clear structural reason for developing it.[72–74] In these patients, a genetic etiology is assumed. Mutations in the gene for the cardiac Na^+ channel (SCN5A) have been recognized to lead to isolated sick sinus syndrome,[56] or the combination of sick sinus syndrome and bradycardia (also known as "tachy-brady syndrome."[59] Mutations in the gene for HCN4, which is in large part responsible for the funny current (I_f) in human nodal tissues, have also been identified in patients with idiopathic SAN disease[38] and in individuals with the combination of long QT, ventricular tachycardia, and SAN disease.[36] These mutations are expected to lead to slowing of diastolic depolarization in nodal cells. Finally, mutations in a Ca^{2+}-handling gene (calsequestrin gene, CASQ2) that lead to autosomal recessive catecholaminergic polymorphic ventricular tachycardia (CPVT) are also associated with SAN dysfunction,[36] postulated to be related to abnormalities in Ca^{2+}-handling in SAN cells, leading to abnormal functioning of the Ca^{2+} clock.

2. **SAN dysfunction and aging**—Aging is associated with SAN dysfunction.[36] It causes a decrease in the overall intrinsic heart rate, and an increase in SAN conduction time.[36,52,75,76] These overt changes in humans appear to be preceded by a period of detectable though clinically silent atrial

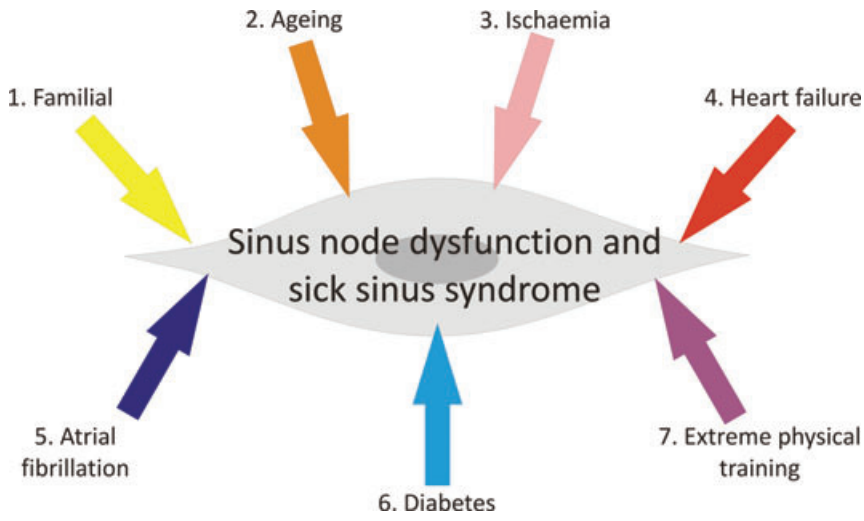

Figure 7. *Schematic diagram illustrating the multifactorial etiology of SAN dysfunction, whereby multiple diverse pathologies can lead to the same disease phenotype.*

remodeling (or "atriopathy") that is particularly apparent around the region of the crista terminalis where the SAN would be expected to lie, and leads to conduction slowing and voltage loss, with evidence of a decrease in SAN reserve.[77] Why this should progress in some patients to clinically overt SAN dysfunction but not in others is currently unclear. The site of the leading pacemaker site does not appear to be affected by age in rabbits and cats,[78] while an inferior shift in leading pacemaker site has been demonstrated in aged rats and humans with SAN dysfunction.[76,77] Sick sinus syndrome is largely a disease of the elderly and its incidence increases in an exponential manner with age.[52] Sick sinus syndrome in the elderly has been previously attributed to fibrosis of the SAN.[79] Aging was first noted to be associated with fibrosis of the SAN as early as 1954,[80] and further work in the 1970s appeared to back this up.[81] More recent work has shown that aging leads to significant remodeling in the extracellular matrix of the SAN of the rat.[76,82] There are, however, conflicting reports on this phenomenon, with a report on the human SAN showing no fibrosis in the elderly.[79] There is more specific evidence for age-related remodeling of ionic currents and ion channels in the SAN. For example, previous authors have documented a decrease in upstroke velocity at the periphery of the SAN with aging, believed to be related to an age-related decrease in I_{Na}.[78] This could lead to exit block from the SAN and an inability of the SAN to drive the surrounding tissue. More recently, a decrease in expression of $Na_v1.5$ has been demonstrated in rats, along with an age-dependent hypertrophy of SAN cells.[76] There is also a decrease in $K_v1.5$ with aging (partly responsible for I_K) in the rat SAN, which could explain the observed increase in action potential duration with aging.[78,83] In the guinea pig, aging causes decreased expression of Cx43 in the vicinity of the SAN, potentially accounting for the observed increase in SAN conduction time and increased incidence of SAN exit block seen with aging.[84] It has also been noted in the guinea pig that $Ca_v1.2$ expression declines during aging,[85] associated with a decrease in SAN electrical activity due to decrease in Ca^{2+} influx via L-type Ca^{2+} channels. This may have implications for the Ca^{2+} clock mechanism of pacemaking. Indeed, the decrease in the intrinsic heart rate during aging in the rat has been attributed to a decrease in the Ca^{2+} clock as a result of a decrease in the expression of RyR2.[86] It has been suggested by some authors that the above noted changes in SAN function associated with aging lead to changes in the anatomy and physiology of the atrial muscle that predispose it to atrial fibrillation.[87]

3. **SAN dysfunction and ischemia**—Coronary artery disease is said to be responsible for as much as one-third of chronic SAN dysfunction.[88] Obviously, bradycardia and sinus arrest are commonly seen in the acute phases of myocardial ischemia and infarction, though this is usually a transient phenomenon in association with acute infarction of the inferior wall associated with altered neurological influences on the heart.[89] However, Brueck et al.[90] surveyed their population of patients presenting for permanent pacemaker implantation due to symptomatic bradycardia, and found significant coronary artery stenoses in 71% of patients, suggesting that covert ischemia may have a very

significant role to play in the development of SAN dysfunction.

4. **SAN dysfunction and heart failure—** Heart failure is associated with SAN dysfunction. Sudden cardiac death accounts for ~50% of the deaths in patients with heart failure.[91] Most of these events are due to ventricular dysrhythmias.[92] However, fatal bradyarrhythmias contribute a significant burden in heart failure; indeed, they account for ~42% of the heart failure sudden deaths in hospital.[91,93,94] The bradyarrhythmias arise in the main because heart failure causes disease of the cardiac conduction system, including sick sinus syndrome[52] and atrioventricular nodal dysfunction.[95] There will also be contributory effects from pharmacological preparations that are negatively chronotropic, but frequently used in heart failure, including β-blockers and digoxin. In a rat model of post-infarction heart failure, we have shown SAN dysfunction (decrease of the intrinsic heart rate and prolongation of the SAN recovery time).[83] During heart failure and atrial tachyarrhythmia in the dog, a down-regulation of HCN channels has been observed in the SAN and this can explain the SAN dysfunction.[96,97] Reduced SAN functional reserve has also been demonstrated in human heart failure patients versus controls—Sanders et al.[92] studied 18 heart failure patients, showing that compared to controls they exhibited prolongation of the intrinsic SAN cycle length and corrected sinus node recovery time; caudal localization of sinus activity both during sinus rhythm and after pacing; prolongation of SAN conduction time; and abnormal, circuitous propagation of the sinus impulse.

5. **SAN dysfunction and atrial fibrillation—** Atrial fibrillation (AF) is well recognized to lead to SAN dysfunction. This was first demonstrated in a chronic tachy-pacing AF model in dogs.[98] Two weeks of 20 Hz pacing in the atria led to increased sinus node recovery time and prolongation of P waves, while maximal heart rate and intrinsic heart rate decreased. A similar prolongation in corrected sinus node recovery time has been noted in patients following electrical cardioversion of chronic AF.[99,100] Much shorter periods of atrial tachy-pacing have subsequently been shown to induce SAN remodeling and dysfunction,[101] though reassuringly this phenomenon appears to be at least in part reversible.[102] SAN remodeling and increased SAN recovery time have also been observed in conditions that predispose to AF, including dysynchronous ventricular pacing[103] and in the longstanding pressure and volume overload associated with chronic atrial septal defect.[97,104]

6. **SAN dysfunction and diabetes—**It is postulated that the microvasculopathy inherent to diabetes causes a higher than normal incidence of SAN dysfunction,[105,106] though finding evidence for why this is in the medical literature is difficult. Wasada et al.[107] presented a case series of four patients who had sick sinus syndrome (sinus arrest with paroxysmal episodes of atrial fibrillation) alongside hyperinsulinemia related to insulin resistance as part of their type 2 diabetes. They postulated that insulin's effect as a stimulator of the cell membrane Na^+/K^+ ATPase could account for the SAN dysfunction seen in these patients with chronic excessively high levels of blood insulin—chronic exposure to hyperinsulinemia, they suggested, could lead to hyperpolarization of SAN myocytes due to depletion of intracellular Na^+. Early experience with the streptozotocin (STZ) model of diabetes in rats showed that soon after diabetes induction, there was a marked decrease in heart rate[108–110]; this partially normalized with insulin treatment. This decrease in nodal function appeared to be intrinsic to the node itself, being present in Langendorff heart preparations and isolated atrial preparations from STZ rats.[111,112] Howarth et al.[113] showed that in the same STZ-induced model of diabetes in rats, there was an increase in SAN cycle length and SAN conduction time. They went on to show that there was upregulation of certain gap junction proteins in the SANs of diabetic rats, and postulated that this may be the cause of the witnessed physiological nodal dysfunction.

7. **SAN dysfunction and extreme physical training—**It is well known that there is a resting bradycardia in athletes. For example, the heart rate of top class, race-fit Tour de France cyclists has been reported to be ~30 beats/min.[114] Previously, this bradycardia has been attributed to high vagal tone.[115] However, there is little or no evidence for this and instead experimental studies have shown that in humans and experimental animals the bradycardia is the result of a decrease in the intrinsic heart rate, i.e., a decrease in the intrinsic pacemaker activity of the SAN.[115] Could this be the result of an ion channel remodeling in the athlete's SAN? The resting bradycardia developed by athletes persists for many years after training has stopped, suggesting that the changes are irreversible.[116] It is also becoming accepted that long-term endurance level exercise is associated with an increase in risk of atrial fibrillation and flutter.[117] Stein et al.[118] demonstrated that trained athletic humans (n = 6) had longer SAN cycle lengths (i.e., slower heart rate), sinus node recovery time, Wenckebach cycle length and effective refractory period of the AV node

compared to control individuals, both at baseline and following blockade of the autonomic nervous system with atropine and propranolol, suggesting that indeed training does cause intrinsic changes to not only the SAN, but also the AVN. On this basis, it seems more likely that endurance training does lead to intrinsic changes in the pacemaking tissues of the heart. However, for this to occur, one appears to at least initially require the presence of a functioning autonomic nervous system—both rats and dogs who were trained following denervation of the heart failed to develop any resting or intrinsic bradycardia.[119,120] The mechanism has been suggested to be that initially, the response to training is mediated by the autonomic nervous system, but over time and with dilatation and hypertrophy of the heart, there is mechano-electrical feedback, leading to intrinsic changes to the SAN and other regions of the cardiac conduction system.[118] Clearly, further studies are required.

Conclusion

The world of the SAN appears to become increasingly fascinating the more we discover about it. Clearly from the above, we have come a long way since the small group of condensed cells were discovered by Keith and Flack. Significant recent developments have enabled us to define the true extent of the SAN, which is markedly greater than previously thought, with the additional existence of a novel paranodal area. We are also coming to appreciate the spectrum of disorders that fall under the umbrella definition of SAN dysfunction, and that though the phenotype of the disorder is often similar, the etiopathogenesis can be markedly different. Finally, and perhaps most excitingly, we are arriving at an appreciation of the fundamental mechanism of pacemaking, which includes both membrane and Ca^{2+} clock hypotheses acting synergistically to lead to a robust yet finely tuned system of dependable pacing function. There do, however, remain some critical vagaries of SAN form and function that will require further elucidation before we can be comfortable that we have a full appreciation of the workings of the intrinsic cardiac pacemaker, and one of the most important factors in making these developments will be accessing human tissue upon which to perform such experiments. Ongoing close collaboration between clinicians and basic scientists will, as ever, be paramount in ensuring that this occurs.

References

1. Keith A. An Autobiography: Sir Arthur Keith. London, UK, Watts and Co, 1950.
2. Keith A, Flack M. The form and nature of the muscular connections between the primary divisions of the vertebrate heart. J Anat Physiol 1907; 41:172–189.
3. Suma K. Sunao Tawara: A father of modern cardiology. Pacing Clin Electrophysiol 2001; 24:88–96.
4. Lewis T. Galvanometric curves yielded by cardiac beats generated in various areas of the auricular musculature: The pacemaker of the heart. Heart 1910; 2:23–46.
5. Wybauw R. Sur le point d'origine de la systole cardiaque dans l'oreillette droite. Arch Int Physiol 1910; 10:78–90.
6. James TN, Sherf L, Fine G, Morales AR. Comparative ultrastructure of the sinus node in man and dog. Circulation 1966; 34:139–163.
7. Masson-Pevet M, Bleeker WK, Mackaay AJ, Bouman LN, Houtkooper JM. Sinus node and atrium cells from the rabbit heart: A quantitative electron microscopic description after electrophysiological localization. J Mol Cell Cardiol 1979; 11:555–568.
8. Opthof T, de Jonge B, Mackaay AJ, Bleeker WK, Masson-Pevet M, Jongsma HJ, Bouman LN. Functional and morphological organization of the guinea-pig sinoatrial node compared with the rabbit sinoatrial node. J Mol Cell Cardiol 1985; 17:549–564.
9. Sanchez-Quintana D, Cabrera JA, Farre J, Climent V, Anderson RH, Ho SY. Sinus node revisited in the era of electroanatomical mapping and catheter ablation. Heart 2005; 91:189–194.
10. Truex RC, Smythe MQ, Taylor MJ. Reconstruction of the human sinoatrial node. Anat Rec 1967; 159:371–378.
11. James TN. Anatomy of the human sinus node. Anat Rec 1961; 141:109–139.
12. Anderson KR, Ho SY, Anderson RH. Location and vascular supply of sinus node in human heart. Br Heart J 1979; 41:28–32.
13. Hoogaars WM, Engel A, Brons JF, Verkerk AO, de Lange FJ, Wong LY, Bakker ML, et al. Tbx3 controls the sinoatrial node gene program and imposes pacemaker function on the atria. Genes Dev 2007; 21:1098–1112.
14. Mommersteeg MTM, Hoogaars WMH, Prall OWJ, de Gier-de Vries C, Wiese C, Clout DEW, Papaioannou VE, et al. Molecular pathway for the localized formation of the sinoatrial node. Circ Res 2007; 100:354–362.
15. Hoogaars WM, Tessari A, Moorman AF, de Boer PA, Hagoort J, Soufan AT, Campione M, et al. The transcriptional repressor Tbx3 delineates the developing central conduction system of the heart. [see comment]. Cardiovasc Res 2004; 62:489–499.
16. Chandler NJ, Greener ID, Tellez JO, Inada S, Musa H, Molenaar P, Difrancesco D, et al. Molecular architecture of the human sinus node: Insights into the function of the cardiac pacemaker. Circulation 2009; 119:1562–1575.
17. Kalman JM, Olgin JE, Karch MR, Hamdan M, Lee RJ, Lesh MD. "Cristal tachycardias": Origin of right atrial tachycardias from the crista terminalis identified by intracardiac echocardiography. J Am Coll Cardiol 1998; 31:451–459.
18. Man KC, Knight B, Tse HF, Pelosi F, Michaud GF, Flemming M, Strickberger SA, et al. Radiofrequency catheter ablation of inappropriate sinus tachycardia guided by activation mapping. J Am Coll Cardiol 2000; 35:451–457.
19. Haqqani HM, Kalman JM. Aging and sinoatrial node dysfunction: Musings on the not-so-funny side. Circulation 2007; 115:1178–1179.
20. Lee RJ, Kalman JM, Fitzpatrick AP, Epstein LM, Fisher WG, Olgin JE, Lesh MD, et al. Radiofrequency catheter modification of the sinus node for "inappropriate" sinus tachycardia. Circulation 1995; 92:2919–2928.
21. Kalman JM, Lee RJ, Fisher WG, Chin MC, Ursell P, Stillson CA, Lesh MD, et al. Radiofrequency catheter modification of sinus pacemaker function guided by intracardiac echocardiography. Circulation 1995; 92:3070–3081.
22. Boyett MR, Honjo H, Kodama I. The sinoatrial node, a heterogeneous pacemaker structure. Cardiovasc Res 2000; 47:658–687.
23. Bromberg BI, Hand DE, Schuessler RB, Boineau JP. Primary negativity does not predict dominant pacemaker location: Implications for sinoatrial conduction. Am J Physiol 1995; 269:H877–H887.
24. Schuessler RB. Abnormal sinus node function in clinical arrhythmias. J Cardiovasc Electrophysiol 2003; 14:215–217.

25. Fedorov VV, Schuessler RB, Hemphill M, Ambrosi CM, Chang R, Voloshina AS, Brown K, et al. Structural and functional evidence for discrete exit pathways that connect the canine sinoatrial node and atria. Circ Res 2009; 104:915–923.
26. Matsuyama T-a, Inoue S, Kobayashi Y, Sakai T, Saito T, Katagiri T, Ota H. Anatomical diversity and age-related histological changes in the human right atrial posterolateral wall. Europace 2004; 6:307–315.
27. Schuessler RB, Boineau JP, Bromberg BI. Origin of the sinus impulse. J Cardiovasc Electrophysiol 1996; 7:263–274.
28. Bozler E. The initation of impulses in cardiac muscle. Am J Physiol 1943; 138:273–282.
29. West TC. Ultramicroelectrode recording from the cardiac pacemaker. J Pharmacol Exp Ther 1955; 115:283–290.
30. Miake J, Marban E, Nuss HB. Biological pacemaker created by gene transfer. Nature 2002; 419:132–133.
31. Irisawa H, Brown HF, Giles W. Cardiac pacemaking in the sinoatrial node. Physiol Rev 1993; 73:197–227.
32. DiFrancesco D. A new interpretation of the pace-maker current in calf Purkinje fibres. J Physiol 1981; 314:359–376.
33. DiFrancesco D. A study of the ionic nature of the pace-maker current in calf Purkinje fibres. J Physiol 1981; 314:377–393.
34. Ludwig A, Zong X, Jeglitsch M, Hofmann F, Biel M. A family of hyperpolarization-activated mammalian cation channels. Nature 1998; 393:587–591.
35. DiFrancesco D, Zipes DP, Jalife J. Pacemaker channels and normal automaticity. In: Zipes DP and Jalife J, eds. Cardiac Electrophysiology: From Cell To Bedside. Philadelphia, PA, Saunders, 2006, pp. 103–111.
36. Ueda K, Nakamura K, Hayashi T, Inagaki N, Takahashi M, Arimura T, Morita H, et al. Functional characterization of a trafficking-defective HCN4 mutation, D553N, associated with cardiac arrhythmia. J Biol Chem 2004; 279:27194–27198.
37. Milanesi R, Baruscotti M, Gnecchi-Ruscone T, DiFrancesco D. Familial sinus bradycardia associated with a mutation in the cardiac pacemaker channel. N Engl J Med 2006; 354:151–157.
38. Schulze-Bahr E, Neu A, Friederich P, Kaupp UB, Breithardt G, Pongs O, Isbrandt D, et al. Pacemaker channel dysfunction in a patient with sinus node disease. J Clin Invest 2003; 111:1537–1545.
39. Nikmaram MR, Boyett MR, Kodama I, Suzuki R, Honjo H. Variation in effects of Cs^+, UL-FS-49, and ZD-7288 within sinoatrial node. Am J Physiol 1997; 272:H2782–H2792.
40. Borer JS, Fox K, Jaillon P, Lerebours G. Antianginal and antiischemic effects of ivabradine, an *If* inhibitor, in stable angina: A randomized, double-blind, multicentered, placebo-controlled trial. Circulation 2003; 107:817–823.
41. Bucchi A, Barbuti A, Baruscotti M, DiFrancesco D. Heart rate reduction via selective 'funny' channel blockers. Curr Opin Pharmacol 2007; 7:208–213.
42. Brown HF, DiFrancesco D, Noble SJ. How does adrenaline accelerate the heart? Nature 1979; 280:235–236.
43. Wainger BJ, DeGennaro M, Santoro B, Siegelbaum SA, Tibbs GR. Molecular mechanism of cAMP modulation of HCN pacemaker channels. Nature 2001; 411:805–810.
44. Herrmann S, Stieber J, Stockl G, Hofmann F, Ludwig A. HCN4 provides a 'depolarization reserve' and is not required for heart rate acceleration in mice. EMBO J 2007; 26:4423–4432.
45. Stieber J, Herrmann S, Feil S, Loster J, Feil R, Biel M, Hofmann F, et al. The hyperpolarization-activated channel HCN4 is required for the generation of pacemaker action potentials in the embryonic heart. Proc Natl Acad Sci USA 2003; 100:15235–15240.
46. Noble D, Denyer JC, Brown HF, DiFrancesco D. Reciprocal role of the inward currents ib, Na and if in controlling and stabilizing pacemaker frequency of rabbit sino-atrial node cells. Proc R Soc Lond B Biol Sci 1992; 250:199–207.
47. Tellez JO, Dobrzynski H, Greener ID, Graham GM, Laing E, Honjo H, Hubbard SJ, et al. Differential expression of ion channel transcripts in atrial muscle and sinoatrial node in rabbit. Circ Res 2006; 99:1384–1393.
48. Mangoni ME, Traboulsie A, Leoni AL, Couette B, Marger L, Le Quang K, Kupfer E, et al. Bradycardia and slowing of the atrioventricular conduction in mice lacking Cav3.1/a-1G T-type calcium channels. Circ Res 2006; 98:1422–1430.
49. Kodama I, Nikmaram MR, Boyett MR, Suzuki R, Honjo H, Owen JM. Regional differences in the role of the Ca^{2+} and Na^+ currents in pacemaker activity in the sinoatrial node. Am J Physiol 1997; 272:H2793–H2806.
50. Platzer J, Engel J, Schrott-Fischer A, Stephan K, Bova S, Chen H, Zheng H, et al. Congenital deafness and sinoatrial node dysfunction in mice lacking class D L-type Ca^{2+} channels. Cell 2000; 102:89–97.
51. Hagiwara N, Irisawa H, Kameyama M. Contribution of two types of calcium currents to the pacemaker potentials of rabbit sino-atrial node cells. J Physiol 1988; 395:233–253.
52. Dobrzynski H, Boyett MR, Anderson RH. New insights into pacemaker activity: Promoting understanding of sick sinus syndrome. Circulation 2007; 115:1921–1932.
53. Lei M, Jones SA, Liu J, Lancaster MK, Fung SS, Dobrzynski H, Camelliti P, et al. Requirement of neuronal- and cardiac-type sodium channels for murine sinoatrial node pacemaking. J Physiol 2004; 559:835–848.
54. Maier SK, Westenbroek RE, Yamanushi TT, Dobrzynski H, Boyett MR, Catterall WA, Scheuer T. An unexpected requirement for brain-type sodium channels for control of heart rate in the mouse sinoatrial node. Proc Natl Acad Sci USA 2003; 100:3507–3512.
55. Lei M, Goddard C, Liu J, Leoni AL, Royer A, Fung SS, Xiao G, et al. Sinus node dysfunction following targeted disruption of the murine cardiac sodium channel gene Scn5a. J Physiol 2005; 567:387–400.
56. Benson DW, Wang DW, Dyment M, Knilans TK, Fish FA, Strieper MJ, Rhodes TH, et al. Congenital sick sinus syndrome caused by recessive mutations in the cardiac sodium channel gene (SCN5A). J Clin Invest 2003; 112:1019–1028.
57. Bezzina C, Veldkamp MW, Van Den Berg MP, Postma AV, Rook MB, Viersma JW, van Langen IM, et al. A single Na^+ channel mutation causing both long-QT and Brugada syndromes. Circ Res 1999; 85:1206–1213.
58. Groenewegen WA, Firouzi M, Bezzina CR, Vliex S, van Langen IM, Sandkuijl L, Smits JP, et al. A cardiac sodium channel mutation cosegregates with a rare connexin40 genotype in familial atrial standstill. Circ Res 2003; 92:14–22.
59. Smits JP, Koopmann TT, Wilders R, Veldkamp MW, Opthof T, Bhuiyan ZA, Mannens MM, et al. A mutation in the human cardiac sodium channel (E161K) contributes to sick sinus syndrome, conduction disease and Brugada syndrome in two families. J Mol Cell Cardiol 2005; 38:969–981.
60. Veldkamp MW, Wilders R, Baartscheer A, Zegers JG, Bezzina CR, Wilde AA. Contribution of sodium channel mutations to bradycardia and sinus node dysfunction in LQT3 families. Circ Res 2003; 92:976–983.
61. Verkerk AO, Wilders R, van Borren MM, Tan HL. Is sodium current present in human sinoatrial node cells? Int J Biol Sci 2009; 5:201–204.
62. Maltsev VA, Lakatta EG. Dynamic interactions of an intracellular Ca^{2+} clock and membrane ion channel clock underlie robust initiation and regulation of cardiac pacemaker function. Cardiovasc Res 2008; 77:274–284.
63. Bogdanov KY, Vinogradova TM, Lakatta EG. Sinoatrial nodal cell ryanodine receptor and Na^+-Ca^{2+} exchanger: Molecular partners in pacemaker regulation. Circ Res 2001; 88:1254–1258.
64. Lancaster MK, Jones SA, Harrison SM, Boyett MR. Intracellular Ca^{2+} and pacemaking within the rabbit sinoatrial node: Heterogeneity of role and control. J Physiol 2004; 556:481–494.
65. Ju YK, Chu Y, Chaulet H, Lai D, Gervasio OL, Graham RM, Cannell MB, et al. Store-operated Ca^{2+} influx and expression of TRPC genes in mouse sinoatrial node. Circ Res 2007; 100:1605–1614.
66. Boyett MR, Inada S, Yoo S, Li J, Liu J, Tellez J, Greener ID, et al. Connexins in the sinoatrial and atrioventricular nodes. Adv Cardiol 2006; 42:175–197.
67. Hagendorff A, Schumacher B, Kirchhoff S, Luderitz B, Willecke K. Conduction disturbances and increased atrial vulnerability in Connexin40-deficient mice analyzed by transesophageal stimulation. Circulation 1999; 99:1508–1515.
68. Kusumoto FM, Goldschlager N. Cardiac pacing. N Engl J Med 1996; 334:89–97.
69. Yabek SM, Jarmakani JM. Sinus node dysfunction in children, adolescents, and young adults. Pediatrics 1978; 61:593–598.
70. Radford DJ, Izukawa T. Sick sinus syndrome. Symptomatic cases in children. Arch Dis Child 1975; 50:879–885.
71. Kugler JD, Gillette PC, Mullins CE, McNamara DG. Sinoatrial conduction in children: An index of sinoatrial node function. Circulation 1979; 59:1266–1276.
72. Ector H, Van Der Hauwaert LG. Sick sinus syndrome in childhood. Br Heart J 1980; 44:684–691.
73. Beder SD, Gillette PC, Garson A Jr, Porter CB, McNamara DG. Symptomatic sick sinus syndrome in children and adolescents as the only manifestation of cardiac abnormality or associated with

unoperated congenital heart disease. Am J Cardiol 1983; 51:1133–1136.
74. Nordenberg A, Varghese PJ, Nugent EW. Spectrum of sinus node dysfunction in two siblings. Am Heart J 1976; 91:507–512.
75. Zipes DP, Jalife J. Sinus node dysfunction: Pathophysiology, clinical features, evaluation and treatment. In: Zipes DP and Jalife J, eds. Cardiac Electrophysiology: From Cell to Bedside, 5th Ed. Philadelphia, PA, Saunders, 2009.
76. Yanni J, Tellez JO, Sutyagin PV, Boyett MR, Dobrzynski H. Structural remodelling of the sinoatrial node in obese old rats. J Mol Cell Cardiol 2010; 48:653–662.
77. Kistler PM, Sanders P, Fynn SP, Stevenson IH, Spence SJ, Vohra JK, Sparks PB, et al. Electrophysiologic and electroanatomic changes in the human atrium associated with age. J Am Coll Cardiol 2004; 44:109–116.
78. Alings AM, Bouman LN. Electrophysiology of the ageing rabbit and cat sinoatrial node -a comparative study. Eur Heart J 1993; 14:1278–1288.
79. Alings AMW, Abbas RF, Bouman LN. Age-related changes in structure and relative collagen content of the human and feline sinoatrial node: A comparative study. Eur Heart J 1995; 16:1655–1667.
80. Lev M. Aging changes in the human sinoatrial node. J Gerontol 1954; 9:1–9.
81. Davies MJ, Pomerance A. Quantitative study of ageing changes in the human sinoatrial node and internodal tracts. Br Heart J 1972; 34:150–152.
82. Yanni J, Boyett MR, Anderson RH, Dobrzynski H. The extent of the specialized atrioventricular ring tissues. Heart Rhythm 2009; 6:672–680.
83. Tellez JO, Dobrzynski H, Yanni J, Billeter R, Boyett MR. Effect of aging on gene expression in the rat sinoatrial node. J Mol Cell Cardiol 2006; 40:982–982.
84. Jones SA, Lancaster MK, Boyett MR. Ageing-related changes of connexins and conduction within the sinoatrial node. J Physiol 2004; 560:429–437.
85. Jones SA, Boyett MR, Lancaster MK. Declining into failure: The age-dependent loss of the L-type calcium channel within the sinoatrial node. Circulation 2007; 115:1183–1190.
86. Tellez JO, Maczewski M, Sutyagin PV, Mackiewicz U, Yanni J, Atkinson A, Inada S, et al. Ageing-dependent remodelling of ion channel and Ca^{2+} clock genes underlying sinoatrial node pacemaking. Exp Physiol 2010 (provisionally accepted for publication).
87. Dun W, Boyden P. Aged atria: Electrical remodeling conducive to atrial fibrillation. J Interv Card Electrophysiol 2009; 25:9–18.
88. Shaw DB, Linker NJ, Heaver PA, Evans R. Chronic sinoatrial disorder (sick sinus syndrome): A possible result of cardiac ischaemia. Br Heart J 1987; 58:598–607.
89. Rokseth R, Hatle L. Sinus arrest in acute myocardial infarction. Br Heart J 1971; 33:639–642.
90. Brueck M, Bandorski D, Kramer W. Incidence of coronary artery disease and necessity of revascularization in symptomatic patients requiring permanent pacemaker implantation. Med Klin 2008; 103:827–830.
91. Uretsky BF, Sheahan RG. Primary prevention of sudden cardiac death in heart failure: Will the solution be shocking? J Am Coll Cardiol 1997; 30:1589–1597.
92. Sanders P, Kistler PM, Morton JB, Spence SJ, Kalman JM. Remodeling of sinus node function in patients with congestive heart failure: Reduction in sinus node reserve. Circulation 2004; 110:897–903.
93. Stevenson WG, Stevenson LW, Middlekauff HR, Saxon LA. Sudden death prevention in patients with advanced ventricular dysfunction. Circulation 1993; 88:2953–2961.
94. Faggiano P, d'Aloia A, Gualeni A, Gardini A, Giordano A. Mechanisms and immediate outcome of in-hospital cardiac arrest in patients with advanced heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. Am J Cardiol.87:655–657.
95. Muir-Nisbet A. The electrophysiology of the atrioventricular node in normal and failing rabbit hearts – PhD Thesis. University of Glasgow, Glasgow, Scotland, 2008.
96. Yeh YH, Burstein B, Qi XY, Sakabe M, Chartier D, Comtois P, Wang Z, et al. Funny current downregulation and sinus node dysfunction associated with atrial tachyarrhythmia: A molecular basis for tachycardia-bradycardia syndrome. Circulation 2009; 119:1576–1585.
97. Zicha S, Fernandez-Velasco M, Lonardo G, L'Heureux N, Nattel S. Sinus node dysfunction and hyperpolarization-activated (HCN) channel subunit remodeling in a canine heart failure model. Cardiovasc Res 2005; 66:472–481.
98. Elvan A, Wylie K, Zipes DP. Pacing-induced chronic atrial fibrillation impairs sinus node function in dogs. Electrophysiological remodeling. Circulation 1996; 94:2953–2960.
99. Kumagai K, Akimitsu S, Kawahira K, Kawanami F, Yamanouchi Y, Hiroki T, Arakawa K. Electrophysiological properties in chronic lone atrial fibrillation. Circulation 1991; 84:1662–1668.
100. Manios EG, Kanoupakis EM, Mavrakis HE, Kallergis EM, Dermitzaki DN, Vardas PE. Sinus pacemaker function after cardioversion of chronic atrial fibrillation: Is sinus node remodeling related with recurrence? J Cardiovasc Electrophysiol 2001; 12:800–806.
101. Hadian D, Zipes DP, Olgin JE, Miller JM, Hadian D, Zipes DP, Olgin JE, et al. Short-term rapid atrial pacing produces electrical remodeling of sinus node function in humans. J Cardiovasc Electrophysiol 2002; 13:584–586.
102. Hocini M, Sanders P, Deisenhofer I, Jais P, Hsu LF, Scavee C, Weerasoriya R, et al. Reverse remodeling of sinus node function after catheter ablation of atrial fibrillation in patients with prolonged sinus pauses. Circulation 2003; 108:1172–1175.
103. Sparks PB, Mond HG, Vohra JK, Jayaprakash S, Kalman JM. Electrical remodeling of the atria following loss of atrioventricular synchrony: A long-term study in humans. Circulation 1999; 100:1894–1900.
104. Morton JB, Sanders P, Vohra JK, Sparks PB, Morgan JG, Spence SJ, Grigg LE, et al. Effect of chronic right atrial stretch on atrial electrical remodeling in patients with an atrial septal defect. Circulation 2003; 107:1775–1782.
105. Park HW, Cho JG, Yum JH, Hong YJ, Lim JH, Kim HG, Kim JH, et al. Clinical characteristics of hypervagotonic sinus node dysfunction. Korean J Intern Med 2004; 19:155–159.
106. Podlaha R, Falk A. The prevalence of diabetes mellitus and other risk factors of atherosclerosis in bradycardia requiring pacemaker treatment. Horm Metab Res Suppl 1992; 26:84–87.
107. Wasada T, Katsumori K, Hasumi S, Kasanuki H, Arii H, Saeki A, Kuroki H, et al. Association of sick sinus syndrome with hyperinsulinemia and insulin resistance in patients with non-insulin-dependent diabetes mellitus: Report of four cases. Intern Med 1995; 34:1174–1177.
108. Howarth FC, Jacobson M, Shafiullah M, Adeghate E. Effects of insulin treatment on heart rhythm, body temperature and physical activity in streptozotocin-induced diabetic rat. Clin Exp Pharmacol Physiol 2006; 33:327–331.
109. Howarth FC, Jacobson M, Shafiullah M, Adeghate E. Long-term effects of streptozotocin-induced diabetes on the electrocardiogram, physical activity and body temperature in rats. Exp Physiol 2005; 90:827–835.
110. Hicks KK, Seifen E, Stimers JR, Kennedy RH. Effects of streptozotocin-induced diabetes on heart rate, blood pressure and cardiac autonomic nervous control. J Auton Nerv Syst 1998; 69:21–30.
111. Howarth FC, Qureshi MA. Effects of carbenoxolone on heart rhythm, contractility and intracellular calcium in streptozotocin-induced diabetic rat. Mol Cell Biochem 2006; 289:21–29.
112. Kofo-Abayomi A, Lucas PD. A comparison between atria from control and streptozotocin-diabetic rats: The effects of dietary myoinositol. Br J Pharmacol 1988; 93:3–8.
113. Howarth F, Nowotny N, Zilahi E, El Haj M, Lei M. Altered expression of gap junction connexin proteins may partly underlie heart rhythm disturbances in the streptozotocin-induced diabetic rat heart. Mol Cell Biochem 2007; 305:145–151.
114. Estes NA 3rd, Link MS, Cannom D, Naccarelli GV, Prystowsky EN, Maron BJ, Olshansky B, Expert Consensus Conference on Arrhythmias in the Athlete of the North American Society of P, Electrophysiololgy. Report of the NASPE policy conference on arrhythmias and the athlete. J Cardiovasc Electrophysiol 2001; 12:1208–1219.
115. Boyett MR. 'And the beat goes on.' The cardiac conduction system: The wiring system of the heart. Exp Physiol 2009; 94:1035–1049.
116. Baldesberger S, Bauersfeld U, Candinas R, Seifert B, Zuber M, Ritter M, Jenni R, et al. Sinus node disease and arrhythmias in the long-term follow-up of former professional cyclists. Eur Heart J 2008; 29:71–78.
117. Mont L, Elosua R, Brugada J. Endurance sport practice as a risk factor for atrial fibrillation and atrial flutter. Europace 2009; 11:11–17.

118. Stein R, Medeiros CM, Rosito GA, Zimerman LI, Ribeiro JP. Intrinsic sinus and atrioventricular node electrophysiologic adaptations in endurance athletes. J Am Coll Cardiol 2002; 39:1033–1038.
119. Sigvardsson K, Svanfeldt E, Kilbom A. Role of the adrenergic nervous system in development of training-induced bradycardia. Acta Physiol Scand 1977; 101:481–488.
120. Ordway GA, Charles JB, Randall DC, Billman GE, Wekstein DR. Heart rate adaptation to exercise training in cardiac-denervated dogs. J Appl Physiol Respir Environ Exerc Physiol 1982; 52:1586–1590.
121. Li J, Greener ID, Inada S, Nikolski VP, Yamamoto M, Hancox JC, Zhang H, et al. Computer three-dimensional reconstruction of the atrioventricular node. Circ Res 2008; 102:975–985.
122. Boineau JP, Canavan TE, Schuessler RB, Cain ME, Corr PB, Cox JL. Demonstration of a widely distributed atrial pacemaker complex in the human heart. Circulation 1988; 77:1221–1237.
123. Mondal T. Personal Communication. 2009.