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The normal sinus node: What we know now

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Abbreviations
HCN: hyperpolarization activated, cyclic nucleotide gated
ICa_T: transient calcium channels
I_f: I_f current
I_{K1}: inward rectifier potassium channels
I_{Kr}: delayed rectifier potassium channel
NCX: sodium-calcium exchanger
SN: sinus node

ABSTRACT
The sinus node is the physiological pacemaker of the heart. Different pathophysiological conditions lead to a reduction of its function, which is clinically called sinus dysfunction. However, for a better understanding of its disease state, it is necessary to elucidate how it works under normal conditions. New evidences indicate that the automatism of the sinus node is produced by the interaction of the membrane clock and the calcium clock, which gives it a strong character that protects it against malfunctions. Current evidences on cell synchrony within the sinus node are presented, as well as the form of electrical propagation and the source-sink coupling. In addition, recent anatomical and histological findings are described.
Keywords: Pacemaker cells, Cardiac electrophysiology, Blood supply, Sinus node, Biological clocks

El nodo sinusal normal: Lo que ahora sabemos

RESUMEN
El nodo sinusal constituye el marcapasos fisiológico del corazón. Diferentes estados fisiopatológicos conducen a una reducción de su función, lo que es llamado en la clínica, disfunción sinusal. Sin embargo, para la mejor comprensión de su estado de enfermedad se requiere dilucidar cómo opera en condiciones normales. Las nuevas evidencias señalan que el automatismo del nodo sinusal se produce por la interacción del reloj de membrana y el reloj de calcio, lo que le confiere un fuerte carácter que lo protege contra fallas de funcionamiento. Se presentan las evidencias actuales sobre la sincronía celular dentro del nodo sinusal, así como la forma de propagación eléctrica y el acoplamiento fuente-sumidero. Además, se describen recientes hallazgos anatómicos e histológicos.
Palabras clave: Células marcapasos, Electrofisiología cardíaca, Irrigación sanguínea, Nodo sinusal, Relojes biológicos

INTRODUCTION

The sinoatrial or sinus node (SN) is the pacemaker of the heart. The reduction of its ability to create a heart rhythm appropriate for the body's needs (sinus dysfunction) is caused by a number of pathophysiological conditions[1,2]; however, if we want to move towards a better understanding of sinus dysfunction we have to elucidate how the SN operates under normal conditions. Recent findings have dramatically changed our knowledge in

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this area, laying the groundwork for future treatments.

BRIEF OVERVIEW

In the XVIII century Albrecht von Haller provided a convincing argument that the heartbeat originated within the heart itself, without requiring input from the nervous system and in the late XIX century, Walter Gaskell noted that the electrical signal began in the sinus auricle and was then conducted through the atrium and on to the ventricles[1]. Sir Arthur Keith and Martin Flack are credited with the discovery of the sinus node. During a sea expedition Wenckebach suggested Keith to study the sinoatrial junction which he had named ultimus moriens in his experiments. Dr. Keith and medical student Martin Flack observed a compact mass of muscle cells different from the surrounding myocardium in human and a number of mammalian hearts. This reminded Keith of the node described by Tawara[2]. In 1907 they officially published their findings.

THE SINUS NODE

The sinoatrial or sinus node (SN) is the heart's natural pacemaker as it automatically produces electrical activity[3,4]. The SN is located sub-epicardially at the junction of the superior vena cava with the right atrium (**Figure 1**). In humans, according to recent studies, SN is a compact-oblong structure surrounded by fatty tissue[5]. The difficulty of its macroscopic identification in corpses is well known. Nooma *et al.*[6] were able to visualize it in only 1 of 16 hearts studied, but they developed a new method that consisted of removing the epicardium from the right atrium around the sulcus terminalis, combining it with the opening of the superior vena cava. The fatty tissue was subsequently brushed using a solution of 40°C water with a surfactant (Triton X: diluted 1:10 with tap water) to show its location. This allowed for a white structure to be visible in all specimens; best appreciated when placed against a black paper inside the right atrium. Histological observation with Masson's trichrome staining demonstrated that the structure in question was consistent with the SN.

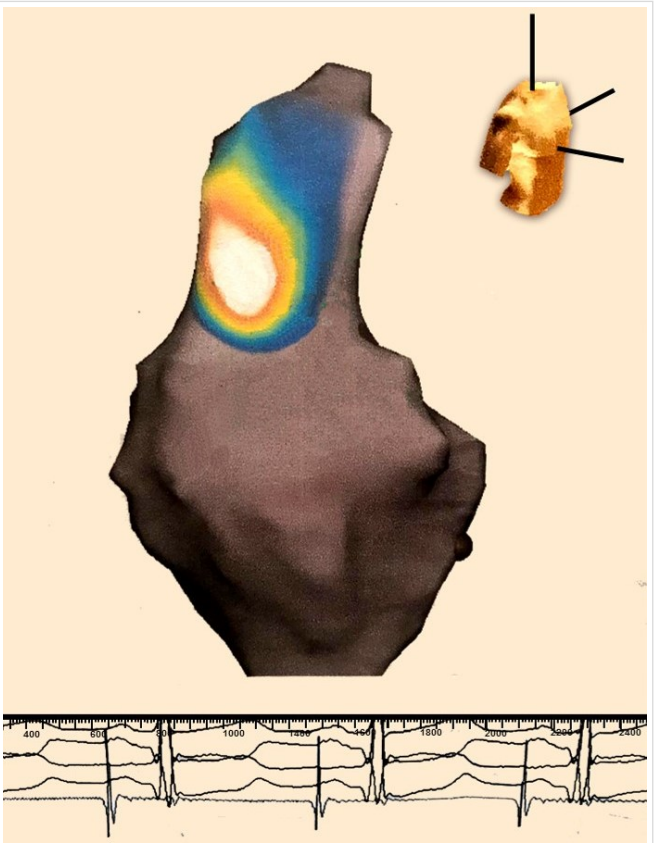

Figure 1. Mapping of the origin of the electrical impulse in the SN using an EnSite navigation system (St. Jude) with Array catheter and non-contact Endocardial Solutions technology (capable of visualizing 3600 points in a single beat). Loops with different colors are obtained (white for the site of origin of the beat in the SN), located in the right atrium, closely related to the superior vena cava's opening. The rest of the colors represent the impulse propagation sequence in the vicinity of the SN. A torso in the upper right side of the figure indicates that images have been taken in right anterior oblique view.

Blood supply

The SN artery is responsible for the blood supply to this structure and has a proximal origin in the right coronary artery in 55-65% of patients, 25-45% in the circumflex artery, and both in 10%[7]. This artery has been typically used to locate the SN, but its retrocaval origin has been proven in up to 47.1% of cases. Therefore, using this branch to identify the anatomy of the SN would not be useful[6]. Recent morphometric studies report that the average maximum diameter of the SN artery in humans is 765.1 ± 229.1 µm and the minimum, 465 ± 152.7 µm, without showing significant differences with the SN of pigs, according to a comparative analysis[5].

Some anatomical studies have suggested that the true supply of the SN starts from its periphery, through a dense arteriolar network that is dichoto-

mized after a variable number of branches, which end in a glomerular capillary network consisting of pericellular glomerular capillary rings[8].

There is evidence of the lack of branches derived from the SN artery which makes its name questionable. Lopes *et al.*[9] stated that "it is the network and not the pacemaker artery that is most responsible for SN nutrition".

Inervación

The SN is densely innervated by adrenergic and cholinergic postganglionic terminals[10]. This neural network originates from the dorsal (containing 26% of all lymph nodes in the heart) and right ventral atrial epicardial subplexes (containing 11% of the human epicardial ganglia)[11]. There is a density of β-adrenergic and muscarinic receptors that is 3 times higher in the SN than in the surrounding atrial myocardium[10,11]. Vagal innervation is lateralized, with the right vagus nerve having a more pronounced effect on the SN as opposed to the left vagus nerve which exerts its action mainly on the atrioventricular node[12].

Histology

The SN is a crescent-shaped structure with long axis parallel to the terminal groove and a mean length of 13.5 mm (range 8–21.5 mm)[13]. Connexin 43 enzyme-linked immunosorbent techniques have shown that NS reaches up to 29.5 mm, suggesting that nodal boundaries based solely on histology are insufficient. Remarkably lower values were found by morphometric measurements of the SN in ten human hearts revealing 1.4-3 mm length and 0.4-1.6 mm width[5].

The sinus node cells are termed P cells (because of their relatively pale appearance under the electron microscope), with a single nucleus (some may be multinucleated) and poorly defined intercalary discs. The pale cellular appearance has also been observed in dogs, horses and pigs[5,14]. Nodal cells are densely packed within fibrous connective tissue, but towards the periphery they may be seen intermingled with ordinary atrial myocytes.

Pale (P cells) vary in size, shape and electrophysiological properties and can be divided into three major classes: 1) elongated spindle-shaped cells (up to 80 μm) that can be multinucleated 2) spindle cells, which have a weak striated body and shorter than the previous ones (up to 40 μm) and 3) spider cells, which show an irregular shape with blunt ends[10]. No multinucleated cells were found in a recent study that analyzed morphometric aspects of SN in humans[5]. Current evidence suggests that the size of these cells is not related to the density of the three main currents involved in pacemaker activity, as previous studies have suggested[15]. Recently, telocytes –a unique type of interstitial cells with telopodes, extremely long but thin prolongations, and dilated segments called podoms– have been described within the SN[16].

Separated from the SN is a paranodal area formed by atrial cells containing a mixture of connexin 43 intermediate expression between the atrial myocardiocytes and those of the SN, which is arranged along the *crista terminalis*, contributing to the expansion of the area with pacing activity. Furthermore, SN extensions greater than 2 mm toward the superior vena cava and the *crista terminalis* have been described. The stem is constituted by the most distal regions of the SN, which is fragmented in more than half of the cases, and forms separate cell clusters that reach as far as the inferior vena cava inflow that appear to contribute to the subsidiary pacemaker bundle. The so-called mosaic effect can be observed in the SN periphery, characterized by the intermingling of nodal and atrial cells with a gradual reduction in the proportion of node cells as it advances towards the atrial tissue[17]. Cell morphology also changes and takes on a transitional appearance between SN and atrial cells, which is known as gradient effect.

As years go by, the sinus cell population tends to decrease until the collagen matrix in which they are immersed predominates; it has also been observed that they increase in volume, which occurs due to cellular hypertrophy[18,19]. The reduction in the number of P cells throughout the aging process undoubtedly contributes to the increased prevalence of sinus dysfunction later in life. Sinus node cells are not detectable in some patients older than 80 years; despite this, they maintain sinus rhythm. It has been postulated that very few of these cells are necessary to maintain sinus rhythm.

ELECTROPHYSIOLOGY

Pacemaker activity

There is no exact anatomical correlation between

compact SN and its functional expression as a pacemaker. Early epicardial mapping studies demonstrated a widely distributed atrial pacemaker complex in human hearts covering a 7.5 x 1.5 cm region centered about the long axis of the sulcus terminalis. More recently, the multicentricity of the SN complex have been confirmed. Multiple origins of the electrical impulse and several exit sites are proposed (**Figure 2**). The beat-to-beat site of sinus activation varies from 0-41 mm, and these findings seem to support the spontaneous P wave variations seen in normal individuals[13]. The higher portions of the SN are associated with higher discharge frequencies and the opposite occurs in the lower portions.

Two-clock theory

Two different mechanisms coexisting at the same time are responsible for pacemaker activity in the SN. The membrane clock (also called voltage-gated) and the calcium clock[17] (**Figure 3**). The molecular elements involved in the functioning of both clocks are located in caveolae-shaped microdomains, rich in caveolin-3, and containing a subpopulation of lipid rafts, or they may be found in a rudimentary T-tubules system closely related to subcellular compartments abundant in type 2 ryanodine receptors (RyR2), which within the SN have only been observed in cells that act as subsidiary pacemakers. This T-tubules system would function as a calcium super-axis[20].

The membrane clock

The term membrane clock refers to the currents that contribute most to early spontaneous depolarization in SN cells. These are: a) funny current (I_f), b) decay of the delayed rectifier K^+ current (I_{kr}) and c) absence of the inward rectifier K^+ current (I_{k1}) in the SN.

Role of I_f

Funny current (I_f) is carried by HCN (hyperpolarization activated, cyclic nucleotide gated) channels located in the plasma membrane. They are non-specific, low conductance cation channels, of which 4 isoforms have been described. In humans, HCN1, HCN2 and HCN4 channels have been found to be expressed in high density in the SN[21]; I_f reversal potential occurs at -20 mV, losing conductance at more positive potentials with with its recovery at hyperpolarized potentials (late phase 3); HCNs are permeable to sodium and potassium ions with large predominance of the former (4:1 ratio), which contributes to depolarization of SN cells.

Two biophysical properties of HCN are indeed intriguing: the first is the lack of potassium selectivity,

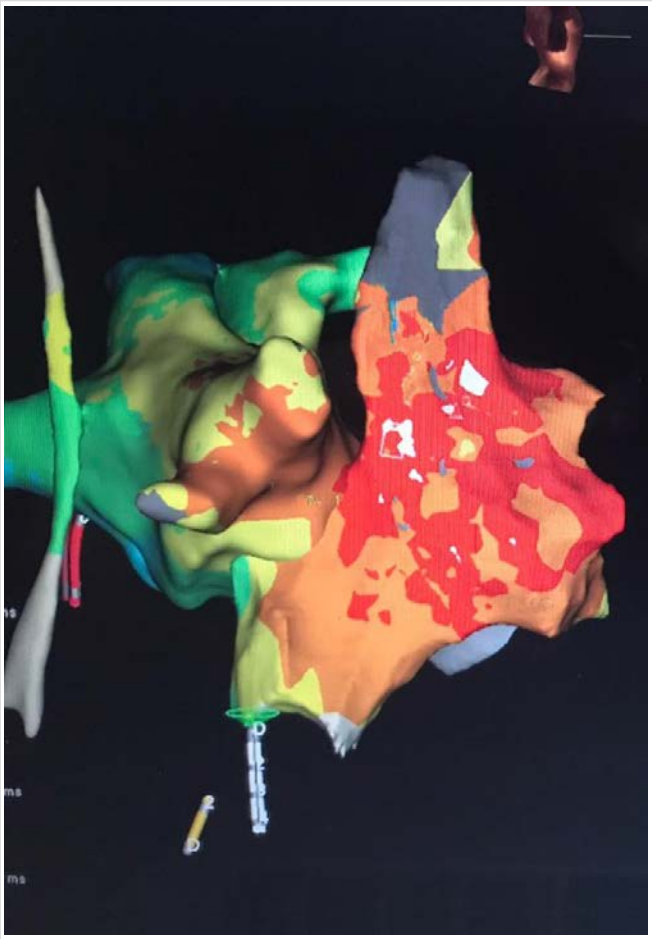

Figure 2. 3D-temporal activation map of the right/left atria obtained using an EnSite (St. Jude) NavX PRECISION navigation system.
Mapping was performed in sinus rhythm, in right anterior oblique view, (white color represents the earliest activation site and is related to SN activity). Note that there are several early sites supporting the existence of more than one origin or exit site. Red color follows in order of precocity indicating depolarization in the lateral wall of the right atrium and crista terminalis; orange, green, blue colors come next and finally, violet tones, giving a sequential sense to the impulse propagation from the right to the left atrium.

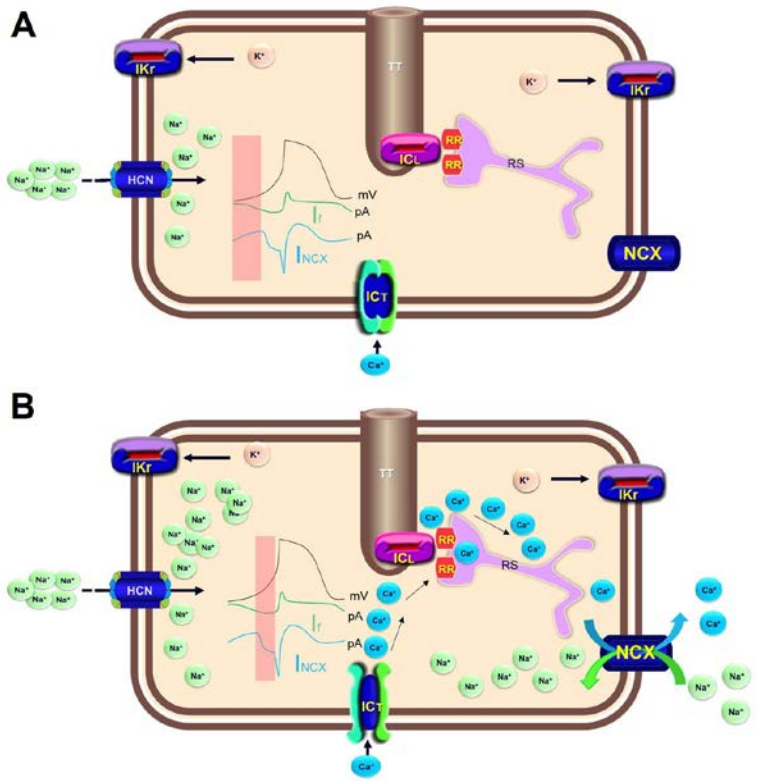

Figure 3. Mechanisms of production of normal automatic activity in the SN through calcium (Ca^{2+})-membrane clocks interaction. **A.** Reveals the first moments of the upward slope durig phase 4 of the action potential, and I_f/NCX current density-plots (indicated by the shaded rectangle). Note the progressive increase in I_f current carried by sodium ions penetrating through HCN channels (left of the figure), giving rise to progressive depolarization unable to bring the membrane to the triggering threshold (membrane clock). **B.** The shaded rectangle highlights the late diastolic acceleration occurring at the end of phase 4 of the action potential and allowing the trigger threshold to be reached. During this period of time, Ca^{2+} ions enter through T-type channels, inducing local Ca^{2+} release events from the SR (Ca^{2+} sparks) through type 2 ryanodine receptors (Ca^{2+} clock). Such Ca^{2+} is exchanged for sodium ions through NCX channels, which powerfully enhances phase 4 depolarization.
HCN, hyperpolarization-activated cyclic nucleotide-gated; IC_L, slow Ca^{2+} channels; IC_T, Ca^{2+} transient channels; I_f, funny current I_f; I_{Kr}, decay of the delayed rectifier K^+ current; mV, millivolts; NCX, sodium-calcium exchanger; pA, picoamperes; RR, ryanodine receptor; SR, sarcoplasmic reticulum; TT, T-tubules.

though containing all the essential amino acids for this function; the second is that voltage gating polarity is reversed, causing an opposite effect to what happens in most voltage gated channels: depolarization causes the gate to close while hyperpolarization causes it to open[22].

Funny current (I_f) plays an important role in the age-related decline in maximum heart rate (mHR). During this stage of life there is responsiveness to sympathetic stimulation, but the maximum heart rate of the heart is decreased, this is attributed in part to a leftward shift of the Boltzmann relation (X axis: membrane voltage Axis Y: gate opening) due to a loss of sensitivity to cAMP (cyclic adenosine monophosphate) that is thought to be related to a protein similar to Trip8b (which modulates HCN1 channels in the brain)[23]. Extrinsic administration of cAMP can reverse these effects.

Although it is known that I_f plays a role in the increase in heart rate after sympathetic stimulation and that certain mutations in HCN4 channels lead to sinus dysfunction, the interrelationship between HCN4 and the autonomic nervous system is still not entirely clear. Kozasa *et al.*[24] studied genetically engineered mice with a novel mutation that induces gain-of-function of HCN4 channels, with potential for 0 to 3-fold reduction in expression levels relative to wild-type genotype mice, and observed that HCN4 overexpression attenuates cervical vagal stimulation-induced bradycardia, but only during β-adrenergic stimulation, suggesting that HCN4 channels attenuate the parasympathetic response of the SN. Although I_f is a critical current in pacemaker activity, it has been shown that some automatic cells in the intercaval region of the rabbit, which lack I_f expression, can retain their ability for spontaneous depolarization; in these cases, the calcium clock seems to have a major contribution[15].

At least three practical applications of I_f are currently recognized[25]:

1. The introduction into clinical practice of ivabradine, a selective I_f blocker and heart rate-reducing agent in ischemic heart disease and heart failure, with no effect on contractility and dromotropism.
2. The discovery of HCN4 mutations, mainly loss-of-function, leading to a bradycardic phenotype, and the description of tachyarrhythmias associated with the only gain-of-function mutation described so far.
3. The development of biological pacemakers with the aim to replace electronic devices. Results in this field dem-onstrate that I_f can transfer its

pacemaking ability to silent cells when implanted in situ by genetic or cellular methods, such as viral vectors.

Role of I_{Kr}

The role played by I_{Kr} is to repolarize the action potential[1] which allows I_f to manifest itself[25], but this —necessarily— has to be accompanied by the absence of I_{K1} current, whose main function is the stabilization of the membrane potential. Monfredi *et al.*[15] described a new relationship between I_f and I_K (delayed rectifier) in pacemaker cells in the rabbit intercaval region, characterized by higher I_f densities when there is greater repolarizing capacity by I_K, which guarantees an increase in automatism.

Role of I_{K1}

Without I_{K1} the SN membrane potential is labile, which is key to early phase 4 depolarization. However, recent studies claim that I_{K1} is determinant to drive ignition of the action potential in autonomic cells, which radically changes some points of the membrane clock theory. Sun *et al.*[26] demonstrated that I_{K1} can promote automaticity in adult ventricular myocardiocytes by upregulation of I_f, and observed that the action of I_{K1} and I_f is enough to induce rhythmic pacemaker oscillations if they are at the appropriate density ratios in non-excitable mouse cells; and termed this model " I_{K1}-induced I_f activation". For their part, Chen *et al.*[27] succeeded in inducing autonomic activity when certain ratios of HCN2 and I_K were achieved in a model of human embryonic kidney cells used as a heterologous expression system. These authors concluded that there are precise interaction dynamics between these two currents and that I_{K1} is necessary to generate automatic activity, including modifications of the firing frequency of pacemaker cells, and extrapolated their findings to the SN, as they claim that in that tissue mechanisms work at very low I_{K1} conductances.

It has also been reported that the sodium background current ($I_{Na,B}$) mediated by non-selective cation channels, whose specific molecular entity has not yet been discovered, could contribute to pacemaker activity in SN and atrioventricular node cells; its conductance, recently measured for the first time, showed values of 3.2 ± 1.2 picosiemens[28].

The calcium clock

It was believed that all automatic activity could be explained by the membrane clock, but it has been found that it operates in mutual entrainment with the calcium clock. It has been shown that calcium ions that function as brief depolarizing pulses known as calcium sparks or local calcium release are released stochastically, spontaneously, repeatedly and synchronously from the sarcoplasmic reticulum. This leakage occurs through type 2 ryanodine channels, increasing its frequency in response to the current provided by transient calcium channels (ICa_T) from the cell membrane, through the calcium-induced calcium release mechanism[29]. The maximum conductance to this cation, mediated by ICa_T, is reached in the last third of phase 4, the calcium sparks they induce generate a subsarcolemmal current that stimulates the Na/Ca exchanger (NCX) to work in the inward direction; NCX extrudes the calcium leaking from the sarcoplasmic reticulum and in return introduces sodium ions that, in addition to that contributed by I_f, produces the final phase 4 slope acceleration, which allows reaching the firing potential (**Figure 3B**).

The robustness of this combined clock system was firmly established with the use of the new selective NCX inhibitor, ORM-10962 and its combination with the well-known I_f inhibitor, ivabradine[30]. The authors demonstrated that individual I_f or NCX blockade did not produce severe bradycardia or instability in their models, since each of these currents compensates for the deficit of the other and makes the SN possess a strong depolarizing capacity. This high safety factor ensures the stability of the pacemaker function. However, Yue *et al.*[1] with the use of a transgenic −NCX-1 knockout (KO)− mice model (cardiac sodium-calcium exchanger), observed that there was an absence of P wave in their electrocardiogram and lack of atrial depolarization demonstrated by intracavitary electrograms. Furthermore, they concluded that the absence of NCX-1 expression disables the ability of the calcium clock to depolarize the membrane, and that I_f was unable of producing spontaneous depolarizations in isolated SN cells, although intermittent action potential firing was generated in the intact SN.

Cell synchrony

Sinus node cells are grouped in bundles with differ-

ent discharge frequencies; however, there is a guiding frequency due to an entrainment mechanism whose mechanistic basis has not been fully elucidated. There is strong doubt that this synchrony can be fully attributed to connexin-mediated intercellular coupling, as only low levels of connexin 45 (predominant isoform) expression have been observed in the central region of the SN, while the others are expressed only in the periphery (connexin 40, 43, and 45)[32], reason for the recent focus on desmosomes which are abundantly expressed and do not seem to play the same role in ventricular myocytes.

In mouse models with loss of desmoplakin function (essential for desmosome assembly) a primary phenotype develops that manifests an increase in the number of resting sinus pauses and changes in the primary activation site within the SN over several beats, and is accompanied by variations in P-wave morphology[33]. Isolation of SN cells allows us to observe three types of cell behavior classified by Kim *et al.*[34] as rhythmic, dysrhythmic and latent. In their work, rhythmic cells showed automatism well coupled in time and space to events of local calcium release from the sarcoplasmic reticulum, while the dysrhythmic and latent cells exhibited partial or total uncoupling in the calcium clock; β -adrenergic stimulation recruits both cell types to fire rhythmically through efficient local calcium release, bringing the two clocks into synchrony. Whether these cells are grouped in different clusters or are responsible for certain heart rate ranges is unknown, but they could be the mechanism that gives rise to the changing localization of the pacemaker within the SN[20].

Tsutsui *et al.*[35] also found SN cells without automatic activity in humans and observed that up to 50% of them began to discharge after exposure to the β-agonist isoprenaline. These cells were more depolarized and exhibited local calcium release uncoupled from the membrane clock.

Nodal conduction and source-sink coupling

The anterograde direction of electrical impulse propagation in the SN is determined by the mosaic effect, the interdigitation of nodal cells with the working atrial myocytes, and the gradient effect[17]. The way the electrical stimulus propagates from the SN to the surrounding atrial myocytes is complex. It has been proposed that this communication could be established through functional barriers, independent structural communication to specialized conduction pathways and true specialized conduction pathways. Recent studies using optical mapping have demonstrated superior, middle and inferior outflow pathways, which correlate with the only sites where there is no isolation by connective tissue and fat in histological studies[36]. Up to five outflow pathways have been well documented using various techniques[37]. Mitrofanova *et al.*[16] have suggested that telocytes may also contribute to impulse conduction.

It is striking that the large mass of atrial myocytes exhibiting resting potentials at -85 mV do not induce, by electrotonic influence, hyperpolarization of the SN cells (holding potential of -60 mV); and that the source-sink mistmatch between the SN (source) and the atrial cardiomyocytes (sink) does not produce a charge dissipation phenomenon that makes it impossible to reach the atrial action potential up to its threshold value. These possible situations seem to be prevented by functional and structural isolation in the form of fibrosis, which confers a high degree of electrical and mechanical confinement[17]. The specialized outflow tracts from the SN, having such slow conduction velocities –due to low levels of connexin 43 expression– result in the accumulation of sufficient charge to excite the atrial myocytes[36]. There is recent evidence that the architectural arrangement within the SN is a strong determinant of its biorhythmicity[38].

The coupling between SN cells and atrial myocytes is now considered to be crucial to establish the mutual entrainment that is established between the different cell clusters within the SN. Another important issue being elucidated is the robust behavior of the SN under adverse conditions. Li *et al.*[39] determined, with the use of infrared optical mapping, 3D histological reconstruction and molecular mapping in explanted human hearts, that the SN protects its function after administration of adenosine (a stimulus used to stress its function) by shifting its pacemaker leader from the central region to higher or lower sites and changing the site of impulse output. Variations in the firing site within the SN also occur in response to sympathetic stimulation (shifts the leading pacemaker toward the head of the SN) or vagal stimulation (favors displacement toward lower sites with lower discharge frequency)[10]. From the molecular point of view, heterogeneity was found in the sensitivity to adenosine determined by different levels of expression of A1R (adenosine receptor 1) and GIRK (G-protein-cou-

pled inward rectifier K^+ channels that determines the $I_{K\text{-}Ado}$ current) proteins, which would be the basis for such behavior. Faced with suppression by atrial overstimulation or atrial fibrillation, protection of the SN occurred by blocking entry into its different conduction pathways. The evidence shown by this study suggests that safety mechanisms against failure are mainly based on modifications of automaticity or conduction, or both; that is, redundant pacing and changes in conduction pathways.

EPILOGUE

We have summarized and presented the latest evidence on blood supply to the SN, the genesis of automatism −explained by the two-clock theory−, the importance of intercellular synchrony, electrical conduction, and electrical coupling between the SN cells and atrial myocytes.

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