

Current aspects of the basic concepts of the electrophysiology of the sinoatrial node

Andrés Ricardo **Pérez-Riera**, MD PhD¹; Raimundo **Barbosa-Barros** MD²; Rodrigo **Daminello-Raimundo**, PhD¹; Luiz Carlos **de Abreu**, PhD¹; Kjell **Nikus**, MD PhD³

¹Laboratório de Metodologia de Pesquisa e Escrita Científica, Faculdade de Medicina do ABC, Santo André, São Paulo, Brazil.

²Coronary Center of the Hospital de Messejana Dr. Carlos Alberto Studart Gomes, Fortaleza, Ceará, Brazil.

³Heart Center, Tampere University Hospital and Faculty of Medicine and Health Technology, Tampere University, Finland.

Andrés Ricardo **Pérez-Riera**: riera@uol.com.br

Raimundo **Barbosa-Barros**: raimundobb@uol.com.br

Rodrigo **Daminello-Raimundo**: rodaminello@yahoo.com.br

Luiz Carlos **de Abreu**: abreu.luizcarlos@gmail.com

Kjell **Nikus**: kjell.nikus@sydansairaala.fi

Corresponding author

Andrés Ricardo **Pérez-Riera**

Rua Sebastião Afonso 885 Zip code: 04417-100 Jardim Miriam, São Paulo-SP, Brazil

Phone/Fax: (55) 11 5621-2390/ e-mail: riera@uol.com.br

Abstract

Cardiac pacemaker cells, also named P-cells (pale cytoplasm, pacemaker, phylogenetically primitive), including cells of the sinoatrial node, are heterogeneous in size, morphology, and electrophysiological characteristics. The exact extent to which these cells differ electrophysiologically in the human heart is unclear, yet it is critical for the understanding of normal cellular function. In this review, we describe major ionic currents and Ca^{2+} clocks acting on Ca^{2+} release in the sarcoplasmic reticulum. We also explain the external regulation of the heart rate controlled by the two branches of the autonomic (involuntary) nervous system: the sympathetic and the parasympathetic nervous system. Vagal stimulus causes bradycardia, rapid and short-duration modulation, and controls rapid responses, and increases heart rate variability. A typical example is constituted by phasic or respiratory sinus arrhythmia, characterized by pronounced vagal activity, more frequent in children and young individuals.

Keywords

Ion channels; sinus node; calcium clock; sarcoplasmic reticulum

Introduction

The cardiac action potential (AP) is a brief change in voltage (membrane potential) across the cell membrane or sarcolemma. This is caused by the movement of charged cations between the inside and outside of the cell, through protein structures called ion channels. The cardiac AP differs from APs found in other types of electrically excitable cells, such as nerves. APs also vary within the heart; this is due to the presence of different ion channels in different cells. Unlike the AP in skeletal muscle cells, the cardiac AP is not initiated by nervous activity. Instead, it arises from a group of specialized cells called cardiac pacemaker cells, also named P cells (pale cytoplasm, pacemaker, phylogenetically primitive), found in high proportion of the right atrium, and constituting the sinoatrial node (SAN); they are heterogeneous in size, morphology, and electrophysiological characteristics and have spontaneous automatic AP generation, roughly 60-100 APs every minute in adults.

SAN dysfunction may be caused by idiopathic degenerative disease, ischemic heart disease, cardiomyopathy, infiltrative disorders, congenital heart disease, electrolyte disturbances and drugs, such as β receptor blockers.

Currents and channels operating on the action potential generation of the sinoatrial node

SAN APs are divided into three phases:

Phase 4 is the spontaneous diastolic depolarization (pacemaker potential) that triggers the AP once the membrane potential reaches a threshold between -40 and -30 mV. **Phase 0** is the depolarization phase of the AP. This is followed by **Phase 3** repolarization. In the SAN, this phase is due to the closure of the L-type calcium channels, preventing inward flux of Ca^{2+} and the opening of the delayed rectifier potassium channels (I_{Kr} , I_{Ks}) [1].

There is no obvious phase 1 and there is no plateau (phase 2) present in pacemaker APs in the SAN (Figure 1).

AP characteristic in the SAN: amplitude: from -60 to +10 mV (70 mV); overshoot: 0-10 mV; duration: 100-300 ms; conduction velocity: 0.05 m/s. Once the cell is completely repolarized at about -60 mV at the end of phase 3 and beginning of phase 4 at maximum diastolic potential (MDP), the cycle is spontaneously restarted. Although SAN pacemaker activity is spontaneously generated by SAN P cells, the rate of this activity can be modified significantly by external factors such as the autonomic influx, hormones, drugs, ions, and ischemia/hypoxia. It is important to note that APs described for SAN P or nodal cells are very similar to those found in the atrioventricular node (AVN). Therefore, APs in the AVN, like the SAN, are determined primarily by changes in slow inward Ca^{2+} and K^+ currents, and do not involve fast Na^+ currents. AVN APs also have intrinsic pacemaker activity produced by the same ion currents as described above for SA nodal cells.

The AVN and Purkinje fibers also have pacemaker activity (spontaneous activation in phase 4) and can therefore spontaneously generate an AP. However, these cells do not usually depolarize spontaneously, because AP production in the SAN is faster. This means that before the AVN or Purkinje fibers reach the threshold potential (TP) for an AP, they are depolarized by the incoming impulse from the SAN [2]. This is called "overdrive suppression" [3]. Pacemaker activity of these cells is vital, as it means that if the SAN fails, the heart can continue to beat, albeit at a lower rate (AVN= 40-60 beats per minute, Purkinje fibers = 20-40 beats per minute). These pacemakers can be life-saving if the SAN fails to activate the heart.

A) **Channels that operate in phase 4:** Under normal conditions, spontaneous automaticity is generally confined to the P-cells of the SAN. In these cells, microelectrode recordings have revealed a spontaneous slow depolarization of the membrane potential during phase 4 of the AP towards the TP. Phase 4 is the spontaneous depolarization (pacemaker potential) that triggers the AP once the membrane potential reaches a threshold between -60 and -40 mV. Pacemaker cells are constantly active. During phase 4, the membrane potential slowly becomes more positive, until it reaches a critical value (around -40 mV; known as the TP) or until it is depolarized by another AP coming from a neighboring cell. The pacemaker potential is thought to be initiated by a group of channels, referred to as hyperpolarization-activated cyclic nucleotide-gated (HCN) channels. These channels open at very negative voltages (i.e. immediately after phase 3 of the previous AP) and allow the passage of both K^+ and Na^+ into the cell. Due to their unusual property of being activated at very negative membrane potentials, these channels (I_f) are referred to as the funny current. The following current/channels operate in phase 4 sequentially (the functional properties of these channels will be discussed more in detail later in “Action potential currents”):

1) **The Pacemaker cardiac “Funny” current/channel, which is abbreviated as I_f :**

Activation during early diastole is the main process underlying generation of the diastolic depolarization, rhythmicity or automatism and spontaneous activity of cardiac pacemaker cells. I_f modulation by autonomic neurotransmitters is responsible for the chronotropic regulation of heart rate (HR). I_f channels have mixed Na^+ and K^+ permeability, activation on hyperpolarization, and very slow kinetics [4]. I_f current, unlike other known currents, is activated from a threshold of -40 to -50mV and reaches maximal activation between -100 and -110 mV, and

it allows ions to enter the cell. It activates slowly upon membrane hyperpolarization (phase 4), and the more negative the membrane potential difference is, the faster the ionic flow. The time constant of the I_f current is one second at -55 mV, shortening by half at -75 mV and rapidly deactivating after membrane depolarization, between +15 and +30 mV, during the AP plateau. Ivabradine, a type 0 antiarrhythmic drug of the new antiarrhythmic classification [5] inhibits I_f , thereby reducing SAN phase 4 pacemaker depolarization rate and HR. Ivabradine may also decrease AVN and Purkinje cells automaticity, thereby increasing in RR intervals [6-10].

- 2) **Transient or T-type Ca^{2+} channel:** As the membrane potential reaches ~ -50 mV, these channels open. The T-type channels are found mainly within the atria and pacemaker cells, but to a lesser degree than L-type channels.

Note: Calcium channels respond to voltage changes across the membrane differently: L-type channels are activated by more positive membrane potentials, take longer time to open and remain open longer than T-type channels. This means that the T-type channels contribute more to depolarization (phase 0) whereas L-type channels contribute to the plateau (phase 2) nonexistent in pacemaker cells. The T-type channels clearly differ from the L-type calcium channels due to their ability to be activated by more negative membrane potentials and to small single channel conductance. They are also non-responsive to calcium antagonist drugs. Cav 3.1 is a T-type calcium channel involved in pacemaking and repetitive firing. The T-type channel is blocked in a selective way by the Ca^{2+} antagonist mibefradil, and other drugs such as bepridil, flunarizine, and pimozide, which bind to the receptor channel in a concentration-dependent fashion, thus blocking the Ca^{2+} cation entrance. Mibefradil decreases HR without affecting contractility

[11]. The great efficacy of bepridil in the treatment of atrial fibrillation or flutter is partially due to the block of this rapid type Ca^{2+} channel [12]. ICa-T is not sensitive to dihydropyridine, and its function is increased with noradrenaline, the α adrenergic agonist phenylephrine, and it is dependent on the ratio of extracellular ATP and endothelin-1.

- 3) **Long-lasting L-type current/channel or ICa^{2+L} :** When the membrane depolarizes to about -40 mV, a second type of Ca^{2+} channel opens. These are the so-called long-lasting, or L-type Ca^{2+} channels. In pacemaker cells (e.g. SAN cells), however, the increase in membrane voltage is mainly due to activation of L-type calcium channels. These channels are also activated by an increase in voltage, either due to the pacemaker potential (phase 4) or an incoming AP. The L-type calcium channels activate towards the end of the pacemaker potential (and therefore contribute to the latter stages of the pacemaker potential). The L-type Ca^{2+} channels are activated more slowly than the Na^+ channels in the ventricular cell, therefore, the depolarization slope in the pacemaker AP waveform is less steep than that in the non-pacemaker AP waveform. The L-type calcium channel (also known as the dihydropyridine channel, or DHP channel) is part of the high-voltage activated family of voltage-dependent calcium channels. Phosphorylation of these channels increases their permeability to calcium and increases the contractility of their respective cardiac myocytes. Thus, by blocking the entry of calcium, calcium channel blockers reduce electrical conduction within the heart, decrease the force of contraction (work) of the muscle cells, and dilate arteries. Dilation of the arteries reduces blood pressure, thereby decreasing the afterload of the left ventricle. This channel is blocked by Class IV calcium antagonists. Calcium channel blockers disrupt the movement of Ca^{2+} through calcium

channels, and they are used as antihypertensive drugs with particular effect on large vessel stiffness. Therefore, they are also suitable for the treatment of systolic hypertension in elderly patients. Calcium channel blockers are also frequently used to alter heart rate, to treat atrioventricular nodal arrhythmias, atrial fibrillation/flutter, to prevent cerebral vasospasm, and to reduce chest pain caused by Prinzmetal angina. Diltiazem is a nondihydropyridine calcium channel blocker that primarily acts on cardiac pacemaker cells and is a Class IV antiarrhythmic. It blocks voltage-dependent L-type calcium channels in the heart to decrease contractility. Diltiazem is slightly less cardioselective than verapamil, but much more so than all the dihydropyridines. Verapamil, which is an L-type calcium channel blocker of the phenylalkylamine class, has been used in the treatment of hypertension, angina, cardiac arrhythmias, and more recently, cluster headaches. It is also effective in preventing migraine, and is a Class IV antiarrhythmic, more effective than digoxin in controlling ventricular rate of atrial fibrillation.

4) Na⁺/Ca²⁺ Exchange: *INaCa* represents the current generated by the action of the sodium-calcium exchanger.

B) Channels that operate in the slope of Phase 0: SAN P cells have a slow fiber profile: its phase 0 is Ca²⁺ dependent, due to an increased Ca²⁺ conductance ($g_{Ca^{2+}}$) through the L-type Ca²⁺ channel. Another mechanism involved in pacemaker potential is known as the Ca²⁺ clock. Ca²⁺ is released spontaneously from the SR into the cell; this is known as a spontaneous Ca²⁺ spark. This increase in Ca²⁺ within the cytoplasm then activates a Na⁺/Ca²⁺ exchanger, which removes one Ca²⁺ from the cell, and exchanges it for 3 Na⁺ ions into the cell (therefore removing a charge of +2 from the cell, but allowing a charge of +3 to enter the cell) therefore increasing the membrane potential.

The Ca^{2+} is later pumped back into the cell via Ca^{2+} channels located on the sarcolemma and SR membrane [13]. The increase in membrane potential produced by these mechanisms, activates T-type Ca^{2+} channels and then L-type Ca^{2+} channels (which open very slowly). These channels allow for a flow of Ca^{2+} into the cell, making the sarcolemmal potential more positive.

C) Operating channels in Phase 3 potassium (K^+) delayed repolarization: As K^+ channels open (increased g_{K^+}), they increase the outward directed, hyperpolarizing K^+ currents. At the same time, the L-type Ca^{2+} channels become inactivated and close, which decreases $g_{\text{Ca}^{2+}}$ and the inward depolarizing Ca^{2+} currents. During repolarization, $g'_{\text{Ca}^{2+}}$ (relative Ca^{2+} conductance) decreases and g'_{K^+} (relative K^+ conductance) increases, which brings membrane potential (E_m) closer toward the equilibrium potential for K^+ , which is about -96 mV). Therefore, the AP in SAN cells are primarily dependent upon changes in Ca^{2+} and K^+ conductance.

Action potential generation

The automatic cells trigger an AP when a TP is reached [14]. The smallest primitive pacemaker cells in the SAN generate the dominant pacemaker potential. P cells have the least polarized maximum diastolic potentials in the range of -50 to -60 mV, the most rapid rates of diastolic depolarization, and the slowest upstroke velocities [15]. Latent pacemakers are concentrated more peripherally in the SAN. These cells are more polarized than the central SAN, with less rapid diastolic depolarization, more abrupt AP transition from diastole to upstroke, and more rapid upstrokes. The depolarizing pacemaker potential is the sum of individual ionic currents, each flowing through a unique

ion channel. The result is an increase in inward current and a decaying outward current. The inward ionic current is due to the flow of cations across the cell membrane with a positive shift in the membrane potential. Subsequent repolarization occurs with flow of positively charged cations out of the cell during phase 3, yielding an outward ionic current and negative shift in the membrane potential [16]. Contemporary molecular biological techniques have further characterized the location and function of the different ion channels in the SAN and the role of each in pacemaker electrophysiology (Figure 2).

The tissue in the central area of the SAN center generates APs with a less negative resting potential, resulting in a single dominant site of pacemaker activity; in contrast, areas in the SAN periphery have a more negative resting potential. A healthy SAN cell has a maximal diastolic potential of -50 to -60 mV. The cardiac AP originates from the SAN, and located in the high right atrium (RA). Its cells depolarize spontaneously and initiate the spontaneous depolarization of APs at a regular rate from the SAN. This rate depends on various conditions, such as atrial stretch and sympathetic activation, but is usually between 60 and 100 bpm at rest in adults' hearts. Myocytes are electrically coupled to each other through gap junctions. These structures consist of connexin molecules and allow direct intercellular communication. Gap junctions do not have a preferential direction of conduction, but because the AP starts in the SAN, it spreads from there through the atria. There is evidence for specialized conduction pathways in the atrium, but their pathophysiologic relevance is still disputed.

Action potential currents

Diastolic depolarization during phase 4 of the SAN AP is the fundamental pacemaker potential and underlies SAN automaticity [17]. It brings the membrane potential to a triggering threshold, thereby initiating the next heartbeat. The interaction of several

individual membrane currents and intracellular currents, some under autonomic influence, execute this depolarization. There are five main ionic currents in the SAN which make up two key ionic systems, the membrane current or “membrane clock”, and the “calcium clock”. These two systems act synergistically in a coupled fashion to produce the SAN AP [18].

The membrane clock is composed of a time- and voltage dependent decay of the outward rectifier potassium current I_K , and voltage-dependent activation of at least three inward currents: The I_f “funny” current, the I_{CaL} -type Ca^{2+} current, and the T-type $I_{Ca^{2+}}$ current. The calcium clock or $I_{Na^+/Ca^{2+}}$ current is initiated by a Ca^{2+} handling mechanism, which is inherently linked with the membrane clock and has an equally important contribution to automaticity [19]. It involves intracellular ryanodine receptor-mediated calcium release from the sarcoplasmic reticulum (SR) resulting in sarcolemmal Na^+/Ca^{2+} exchange and has an important role in regulating sinus HR [20].

Potassium currents

The stable negative resting potential of working myocardium is generated by the repolarizing outward potassium currents I_{K1} and I_K . The strong inward rectification of the I_{K1} limits the outward current during phases maintaining stable the negative resting membrane potential, since K^+ permeability dominates in the resting state. I_{K1} is abundant in the ventricles but scarce in the atria. I_{K1} is absent in the SAN and I_K is less abundant than in the ventricles, allowing the membrane potential to drift upward in early diastole [21]. This renders the SAN cell membrane potential labile with no stable negative resting potential, allowing opposing inward currents, particularly I_f , but also $I_{Na^+Ca^{2+}}$, to depolarize nodal myocytes [22]. Early diastolic depolarization results, with pacemaker cells operating at diastolic potentials between -60 and -30 mV [23]. I_K is the delayed

rectifier current and has rapid and slow components, I_{Kr} and I_{Ks} , carried by the K^+ channels, hERG and KvLQT1, respectively. I_{Kr} and I_{Ks} are activated during the AP and are responsible for repolarization of the myocyte at the end of the AP by increasing K^+ permeability, thereby returning the trans-membrane potential close to the K^+ equilibrium potential. In the SAN, however, the rectifier potassium current deactivates and decays after the AP is complete [24]. As a result, in diastole, the membrane potential drifts toward the more positive equilibrium potentials of other ions (Na^+ , Ca^{2+} , and Cl^-) allowing these inward currents to depolarize the SAN myocytes. I_K decay contributes much of the earliest part of the pacemaker potential.

Sodium current

The inward Na^+ current I_{Na} is abundant in working myocardium and is carried by the Nav1.5 channel encoded by the *SCN5A* gene. It is responsible for the fast upstroke of the AP in working myocardium but is mostly absent in the center of the SAN, explaining the slower upstroke of the pacemaker AP. Transitional cells at the SAN periphery may contain some Nav1.5 channels, albeit at low levels, providing some excitatory Na^+ current in this region, and explaining the more rapid upstrokes in the SAN periphery than in the center [25].

The funny current I_f

The funny current I_f is a nonspecific mixed inward cation current carried by Na^+ and K^+ ions [26]. This positive current is a major contributor to early diastolic depolarization [27]. I_f passes through membrane bound channels encoded by the gene HCN4 [22]. This gene is highly expressed in central SAN cells, and to a lesser degree, in peripheral latent pacemakers. It is specifically activated at hyperpolarized membrane potentials and is

slowly activated early in phase 4 diastole when the cell is hyperpolarized at its most negative membrane potential. There are four isoforms of the gene HCN. HCN1 and HCN4 are the predominant cardiac isoforms in the SAN. These two genes are expressed in abundance in the SAN but absent in working atrial myocardium, suggesting the importance of I_f in pacemaker activity. Selective blockers of I_f such as ivabradine have been shown to slow the sinus rate in human subjects by decreasing the slope of the pacemaker potential [22, 28]. I_f ion channels are voltage gated, being hyperpolarization activated at membrane potentials below -60 mV. I_f channels are also cyclic-nucleotide gated. Cyclic AMP signaling modulates their electrophysiological properties rendering them responsive to adrenergic and cholinergic stimulation. HCN channels and I_f are thus pivotal in the autonomic regulation of HR. Following sympathetic stimulation, β_1 -adrenoceptor activation of adenylate cyclase increases intracellular cAMP. This binds to and increases opening of the HCN channel with an increase in I_f current. The result is a rise in HR.

Vagal stimulation reduces cAMP and the I_f current, resulting in slowing of the sinus rate. Block of I_f increases HR variability, indicating that I_f may be important in HR stabilization [29]. This is important since decreased HR variability is associated with an increased risk of arrhythmia and sudden cardiac death [30].

Calcium currents

Two separate Ca^{2+} currents have been recorded from SAN cells, the transient type ($\text{I}_{\text{Ca}^{2+}\text{T}}$) and the long-lasting type ($\text{I}_{\text{Ca}^{2+}\text{L}}$) [31]. Voltage-gated calcium channels are activated by the rising membrane potential late during the pacemaker potential, producing two excitatory calcium currents; the transient T-type current $\text{I}_{\text{Ca}^{2+}\text{T}}$ and the long-lasting L-type current $\text{I}_{\text{Ca}^{2+}\text{L}}$ [32, 33]. $\text{I}_{\text{Ca}^{2+}\text{T}}$ has an activation threshold more negative than

-40 mV and is therefore activated earlier in diastolic depolarization than the long-lasting L-type current. $I_{Ca^{2+}T}$ flows through the Cav3.1 and Cav3.2 channels, which are found in abundance in cardiac myocytes displaying automaticity, including SAN myocytes.

$I_{Ca^{2+}L}$ is responsible for the slow AP upstroke in the SAN. It activates late due to its activation threshold being more positive than -40 mV. At TP, phase 0 of the AP is triggered and L-type voltage-gated Ca^{2+} channels open, allowing large-scale depolarization of the cell via Ca^{2+} influx. The current is slower and of much lower amplitude than the Na^+ current of working myocardium, resulting in a slow AP upstroke and slow conduction within the SAN. $I_{Ca^{2+}L}$ is the trigger for Ca^{2+} release by the SR, and is therefore the trigger for contraction in all cardiac cells. $I_{Ca^{2+}L}$ in the SAN flows through the Ca^{2+} channel, Cav1.3. $I_{Ca^{2+}L}$ responds to autonomic influences.

The calcium clock $I_{Na^+ Ca^{2+}}$ exchange current

In the second part of diastolic depolarization, or phase 4, of the SAN-AP, Ca^{2+} is spontaneously released from the SR into the cytosol. These “sparks” or local Ca^{2+} releases leave the SR via ryanodine receptor calcium channels (RYRs). The resulting rise in intracellular calcium concentration activates the Na^+/Ca^{2+} exchanger cell membrane pump (NCX) [34]. NCX exchanges one intracellular Ca^{2+} ion for three extracellular Na^+ ions, generating the net positive inward Na^+/Ca^{2+} exchange current $I_{Na^+/Ca^{2+}}$ (also known as INCX). Activation of I_{NaCa} in late diastole is responsible for the final exponential phase of the pacemaker potential. In a process known as “ Ca^{2+} -induced Ca^{2+} release,” Ca^{2+} extrusion from the SR via RYR increases in response to Ca^{2+} entry into the cell. Cellular entry of Ca^{2+} via $I_{Ca^{2+}L}$ and $I_{Ca^{2+}T}$ is activated by the rising membrane potential initiated by the membrane clock [18]. The result of these processes is whole-cell Ca^{2+} sweeping across the cell, with myofilament contraction. The

combination of the above intracellular Ca^{2+} cycling processes, in the late stage of depolarization of SAN cells, is known as the calcium clock [19]. The clock is driven by high levels of phosphorylation of Ca^{2+} -cycling proteins and is modulated by adrenergic and cholinergic stimulation. Regulation by these cAMP-driven pathways of the autonomic nervous system gives the calcium clock an important role in SAN rate determination [19]. SR Ca^{2+} is replenished by reuptake of Ca^{2+} via sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA), the speed of which is enhanced by adrenergic and slowed by cholinergic receptor activation. Store-operated Ca^{2+} channels at the cell surface membrane also replenish SR Ca^{2+} . These channels may set the frequency of the Ca^{2+} clock by regulating the amount of Ca^{2+} within the SR [35].

In contrast to ventricular and atrial cells, the SAN myocyte expresses virtually no inward rectifier K^+ current (I_{K1}), contributing to an AP without a stable resting potential. Instead, upon reaching a maximum diastolic potential (≈ -60 mV), the SAN AP undergoes spontaneous depolarization, eventually reaching the threshold for generation of another AP. The I_f current generates a depolarizing current during this phase together with the $\text{Na}^+/\text{Ca}^{2+}$ exchanger in response to local Ca^{2+} release from the SR ryanodine receptor Ca^{2+} release channels [36, 37]. Eventually, the membrane potential reaches the threshold (around -50 mV) for activation of transient or T-type Ca^{2+} channels that generate a depolarizing current to accelerate spontaneous depolarization. L-type Ca^{2+} channels activation triggers the SAN AP upstroke, that is much slower compared to atrial or ventricular APs because of the relatively low expression of voltage-gated Na^+ channel (Na_v). While Na_v does not contribute significantly to the SAN AP upstroke (especially in the central SAN region), Na_v expression in atrial tissue surrounding the SAN (and SAN periphery itself) helps to ensure robust pacemaking. A host of voltage-gated K^+ channels govern SAN AP repolarization. The transient outward K^+ current is responsible for the

early repolarization, while ultrarapid (I_{Kur} , $K_v1.5$), rapid (I_{Kr} , ERG), and slow (I_{Ks} , K_vLQT1) delayed rectifier K^+ currents determine late repolarization phase along with the maximal diastolic potential (I_{Kr} , in particular, in the absence of I_{K1}). Currents such as ATP- and acetylcholine- sensitive K^+ currents ($I_{K,ATP}$, $I_{K,Ach}$, respectively) impart dynamic responsiveness of the SAN to parasympathetic and metabolic factors.

Regulation of the heart rate by the autonomic nervous system

The speed of AP production in pacemaker cells is affected, but not controlled by the autonomic nervous system (ANS). The sympathetic nervous system increases HR (positive chronotropy), by decreasing the time to produce an AP in the SAN. Nerves from the spinal cord release noradrenaline, which binds to and activates β_1 adrenoceptors on the pacemaker cell membrane. This activates the Gs-protein (s for stimulatory). Activation of this G-protein leads to increased levels of cAMP in the cell (via the cAMP pathway). cAMP binds to the HCN channels, increasing the I_f current and therefore increasing the rate of depolarization, during the pacemaker potential. The increased cAMP also increases the opening time of L -type calcium channels, increasing the Ca^{2+} current through the channel, speeding up phase 0.

The parasympathetic nervous system decreases HR (negative chronotropy), by increasing the time taken to produce an AP in the phase 4 of SAN. The vagus nerve (historically cited as the pneumogastric nerve) is the tenth cranial nerve or CN X, and interfaces with the parasympathetic control of the heart, lungs, and digestive tract. The vagus nerves are paired but are normally referred to as a single one. It is the longest nerve of the autonomic nervous system in the human body. The ending part of vagus nerve is known as spinal accessory nucleus., that begins in the brain and travels to the SAN, releasing acetylcholine (ACh), which binds to the M2 muscarinic receptor located on the outside of the

pacemaker cell. This activates a Gi-protein (I for inhibitory), which is made up of 3 subunits (α , β and γ) which, when activated, are separated from the receptor. The β and γ subunits activate a special set of K^+ channels, increasing K^+ flow out of the cell and decreasing membrane potential, meaning that the pacemaker cells take longer to reach their threshold value [38]. The Gi-protein also inhibits the cAMP pathway therefore reducing the sympathetic effects caused by the spinal nerves [39] (Figure 3) [40].

β receptor blockers decrease the HR by binding to adrenoreceptors, while atropine increases the HR through its antimuscarinic effect.

Vagal stimulus causes bradycardia, rapid and short-duration modulation, and controls rapid responses, and increases heart rate variability. A typical example is constituted by phasic or respiratory sinus arrhythmia, characterized by a pronounced vagal effect, more frequent in children and young individuals. This type of sinus automatism is related to respiratory phases, with slower periods coinciding with the end of expiration, and the more rapid ones with the end of inspiration. A P-P or R-R variation of ≥ 120 ms or $>10\%$ is typical for sinus arrhythmia.

Gap junctions

Gap junctions are responsible for the electrical coupling between cardiac myocytes and the spread of the AP through the heart [41]. They are comprised of connexins. Connexin 43, which is responsible for the high conduction velocity of the AP in the working myocardium, is not expressed in the center of the SAN. Instead, connexin 45 is expressed in the SAN, forming small conductance gap junctions. Consequently, the myocytes in the center of the SAN are poorly electrically coupled [42]. In the periphery of the SAN, the electrical coupling improves with expression of connexin 43, and interdigitations between

the SAN and the atrial myocytes could facilitate the propagation of the AP from the SAN into the atrial muscle.

Animal studies have demonstrated two different atrial pacemaker cell types: stellate or spiderlike cells and spindle-shaped cells [43]. The spider cells demonstrated faster spontaneous depolarization than the spindle cells. These cells also expressed distinct connexin phenotypes, which could contribute to the non-uniform conduction properties seen in this tissue [44].

Animal studies indicate that proper function of pacemaker cells requires desmosomes, and that loss of anchorage between cells may be a contributing factor to sinus node disease [45]. This also indicates that heart rate variability can be independent of autonomic nervous system stimulation.

Anatomical aspects

The human right atrium and SAN anatomy is complex. Atrial activation maps have demonstrated a widely distributed atrial pacemaker complex in the human heart, and it was proposed that faster impulse rates are initiated by more superiorly positioned atrial pacemakers and slower rates by more inferiorly positioned sites [46]. Although these anatomical and physiological aspects are disputed, it seems clear that pacemaker activity is not restricted to the anatomic SAN located at the superior margin of the cavoappendicular junction [41]. Schuessler et al observed both spider- and spindle-shaped cells. They also observed that the spider-shaped cells are single cells having a single nucleus [47]. Verheijck et al have made the same observation, and they subdivided the spindle-shaped cells into two classes based on size [48]. Schuessler et al found that spider cells have an average of four projections (range 3 to 7) off the central body with a

maximum width of 7 to 9 μm . The cell's maximum length, measured from the ends of the projections, was 92 to 102 μm [47].

The spindle cells have a narrower central body, with maximum width of 5 to 7 μm and average length of 115 to 130 μm . Spider cells have a capacitance of 29 to 32 pF, compared with 32 to 38 pF for the spindle cells. Both cell types have similar membrane resistances, ranging from 1.2 to 1.5 $\text{G}\Omega$ [47].

The structural and functional connections between the SAN and the right atrium in humans is not fully known. Animal work demonstrated that the SAN 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 [41].

Post-mortem anatomical studies of human heart specimens showed that specialized SAN cells intermingled with ordinary atrial myocytes without a discrete fibrous border [49]. This could cause inhomogeneous refractoriness. On the other hand, normal function of the SAN is dependent on protection from the hyperpolarizing influence of the surrounding atrial muscle [50]. This can probably be achieved by unique anatomical and electrophysiological properties of these structures, such as poor electrical coupling within the SAN, a leading pacemaker site in the center of the SAN and high density of I_f and I_{Na} in the periphery of the SAN [50]. Also the fact that the conduction velocity of the AP in the center of the SAN is slow, results in electrical insulation from the surrounding atrial muscle [42].

Summary

The SAN is a complex tissue and its function depends on this complexity. Proper SAN function is dependent on proper function of the P cells, membrane and clock currents to

ensure an organized spread of the AP from the SAN to the atria, with further electrical activation of the atrioventricular node and the ventricles.

Initiation of the sinus impulse is a complex and dynamic process that involves the SAN and extranodal pacemakers composed of multiple cell types, controlled by different local and reflex mechanisms. The node is structurally organized with a putative network of conduction bundles that exit at a limited number of sites. The result is an impulse origin that is multicentric and dynamic, changing with modifying physiological conditions. It is reflected in the rate-related changes observed in the P-wave morphology in the standard surface electrocardiogram. This model of impulse origin also provides a framework for the understanding of sinus tachycardia, sinus bradycardia, sinus arrhythmia, premature atrial contractions, ectopic atrial tachycardias, and sinus node reentry.

Disclosure

None.

References

1. Clark RB, Mangoni ME, Lueger A, *et al.* A rapidly activating delayed rectifier K⁺ current regulates pacemaker activity in adult mouse sinoatrial node cells. *Am J Physiol Heart Circ Physiol.* 2004;286:H1757-66. doi: 10.1152/ajpheart.00753.2003]
2. Tsien RW, Carpenter DO. Ionic mechanisms of pacemaker activity in cardiac Purkinje fibers. *Fed Proc.* 1978;37:2127-31.
3. Vassalle M. The relationship among cardiac pacemakers. Overdrive suppression. *Circ Res.* 1977;41:269-77.
4. Brown HF, DiFrancesco D, Noble SJ. How does adrenaline accelerate the heart? *Nature.* 1979;280:235-6.
5. Lei M, Wu L, Terrar DA, *et al.* Modernized Classification of Cardiac Antiarrhythmic Drugs. *Circulation.* 2018;138:1879-96. doi: 10.1161/CIRCULATIONAHA.118.035455]
6. Biel M, Wahl-Schott C, Michalakakis S, *et al.* Hyperpolarization-activated cation channels: from genes to function. *Physiol Rev.* 2009;89:847-85. doi: 10.1152/physrev.00029.2008]
7. Capel RA, Terrar DA. The importance of Ca²⁺-dependent mechanisms for the initiation of the heartbeat. *Front Physiol.* 2015;6:80. [PMID: 4373508 doi: 10.3389/fphys.2015.00080]
8. Lei M, Zhang H, Grace AA, *et al.* SCN5A and sinoatrial node pacemaker function. *Cardiovasc Res.* 2007;74:356-65. doi: 10.1016/j.cardiores.2007.01.009]
9. Mangoni ME, Couette B, Marger L, *et al.* Voltage-dependent calcium channels and cardiac pacemaker activity: from ionic currents to genes. *Prog Biophys Mol Biol.* 2006;90:38-63. doi: 10.1016/j.pbiomolbio.2005.05.003]

10. Mangoni ME, Nargeot J. Genesis and regulation of the heart automaticity. *Physiol Rev.* 2008;88:919-82. doi: 10.1152/physrev.00018.2007]
11. Uchino T, Lee TS, Kaku T, *et al.* Voltage-dependent and frequency-independent inhibition of recombinant Cav3.2 T-type Ca²⁺ channel by bepridil. *Pharmacology.* 2005;74:174-81. doi: 10.1159/000085329]
12. Palatini P. Heart rate as a risk factor for atherosclerosis and cardiovascular mortality: the effect of antihypertensive drugs. *Drugs.* 1999;57:713-24. doi: 10.2165/00003495-199957050-00004]
13. Joung B, Chen PS, Lin SF. The role of the calcium and the voltage clocks in sinoatrial node dysfunction. *Yonsei Med J.* 2011;52:211-9. [PMID: 3051220 doi: 10.3349/ymj.2011.52.2.211]
14. Robinson RB, Difrancesco D. Sinoatrial node and impulse initiation. In: Spooner PM, Rosen M, editors. *Foundations of cardiac arrhythmias: basic concepts and clinical approaches.* New York: Marcel Dekker; 2001.
15. Bleeker WK, Mackaay AJ, Masson-Pévet M, *et al.* Functional and morphologic organization of the rabbit sinus node. *Circulation Research.* 1980;46:11-22.
16. Vassalle M, Yu, H., Cohen, I. S., Zipes, D. P., Jalife, J. (). . . . Pacemaker channels and cardiac automaticity. In: Zipes DP, Jalife J, editors. *Cardiac electrophysiology: from cell to bedside.* Second ed. Philadelphia: W B Saunders Co; 1995.
17. Bozler E. The initiation of impulses in cardiac muscle. *Am J of Physiol.* 1943;138:273-82.
18. Maltsev VA, Lakatta EG. Dynamic interactions of an intracellular Ca²⁺ clock and membrane ion channel clock underlie robust initiation and regulation of cardiac pacemaker function. *Cardiovasc Res.* 2008;77:274-84. doi: 10.1093/cvr/cvm058]

19. Maltsev VA, Lakatta EG. Normal heart rhythm is initiated and regulated by an intracellular calcium clock within pacemaker cells. *Heart Lung Circ.* 2007;16:335-48. [PMID: 2078332 doi: 10.1016/j.hlc.2007.07.005]
20. Monfredi O, Maltsev VA, Lakatta EG. Modern concepts concerning the origin of the heartbeat. *Physiology (Bethesda).* 2013;28:74-92. [PMID: 3768086 doi: 10.1152/physiol.00054.2012]
21. Chandler NJ, Greener ID, Tellez JO, *et al.* Molecular architecture of the human sinus node: insights into the function of the cardiac pacemaker. *Circulation.* 2009;119:1562-75. doi: 10.1161/CIRCULATIONAHA.108.804369]
22. DiFrancesco D, Borer JS. The funny current: cellular basis for the control of heart rate. *Drugs.* 2007;67 Suppl 2:15-24. doi: 10.2165/00003495-200767002-00003]
23. Shibata EF, Giles WR. Ionic currents that generate the spontaneous diastolic depolarization in individual cardiac pacemaker cells. *Proc Natl Acad Sci U S A.* 1985;82:7796-800. [PMID: 391421 doi: 10.1073/pnas.82.22.7796]
24. Irisawa H, Brown HF, Giles W. Cardiac pacemaking in the sinoatrial node. *Physiol Rev.* 1993;73:197-227. doi: 10.1152/physrev.1993.73.1.197]
25. Dobrzynski H, Boyett MR, Anderson RH. New insights into pacemaker activity: promoting understanding of sick sinus syndrome. *Circulation.* 2007;115:1921-32. doi: 10.1161/CIRCULATIONAHA.106.616011]
26. DiFrancesco D. A new interpretation of the pace-maker current in calf Purkinje fibres. *J Physiol.* 1981;314:359-76. [PMID: 1249439 doi: 10.1113/jphysiol.1981.sp013713]
27. Verkerk AO, Wilders R, van Borren MM, *et al.* Pacemaker current (I_f) in the human sinoatrial node. *Eur Heart J.* 2007;28:2472-8. doi: 10.1093/eurheartj/ehm339]

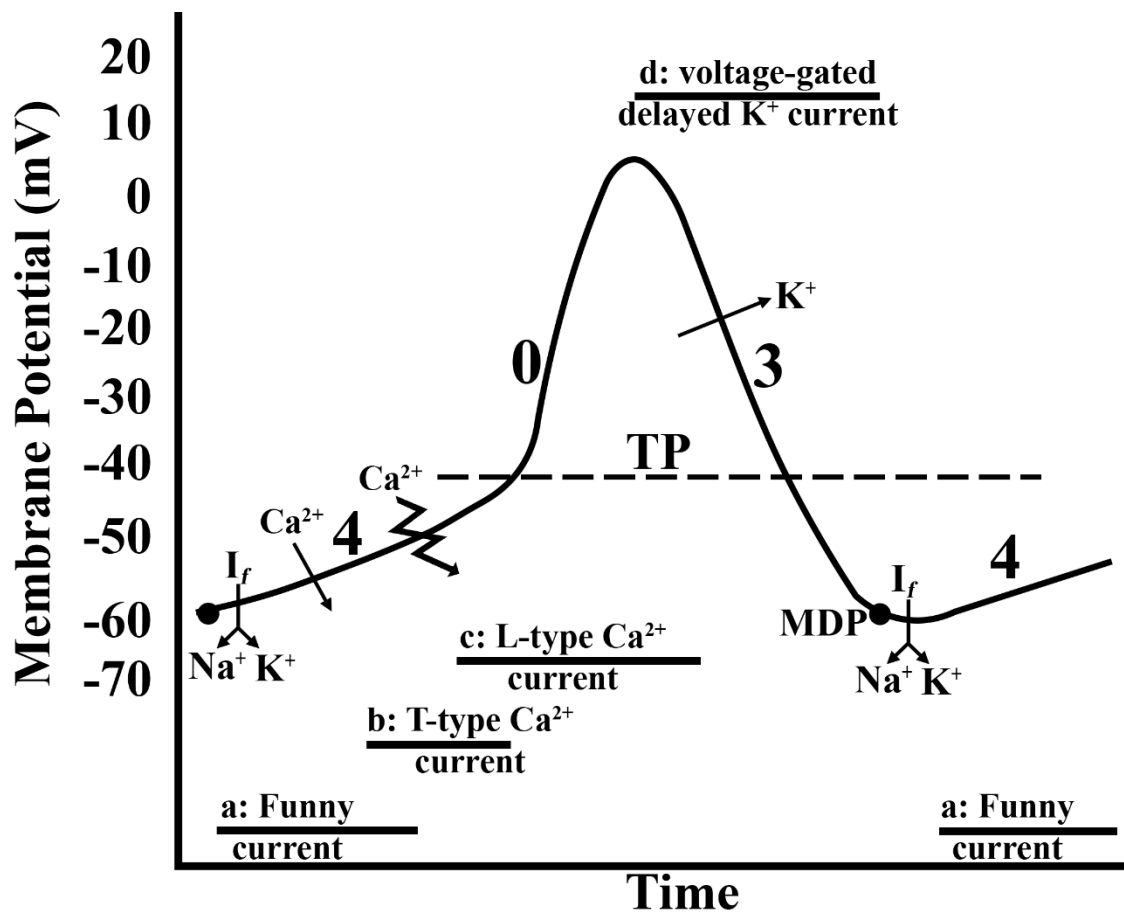
28. Bucchi A, Barbuti A, Baruscotti M, *et al.* Heart rate reduction via selective 'funny' channel blockers. *Curr Opin Pharmacol.* 2007;7:208-13. doi: 10.1016/j.coph.2006.09.005]
29. Noble D, Denyer JC, Brown HF, *et al.* Reciprocal role of the inward currents I_b , I_{Na} and I_{f} in controlling and stabilizing pacemaker frequency of rabbit sino-atrial node cells. *Proc Biol Sci.* 1992;250:199-207. doi: 10.1098/rspb.1992.0150]
30. Lombardi F, Makikallio TH, Myerburg RJ, *et al.* Sudden cardiac death: role of heart rate variability to identify patients at risk. *Cardiovasc Res.* 2001;50:210-7. doi: 10.1016/s0008-6363(01)00221-8]
31. Dobrzynski H, Li J, Tellez J, *et al.* Computer three-dimensional reconstruction of the sinoatrial node. *Circulation.* 2005;111:846-54. doi: 10.1161/01.CIR.0000152100.04087.DB]
32. 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;395:233-53. [PMID: 1191991 doi: 10.1113/jphysiol.1988.sp016916]
33. Kodama I, Nikmaram MR, Boyett MR, *et al.* Regional differences in the role of the Ca^{2+} and Na^{+} currents in pacemaker activity in the sinoatrial node. *Am J Physiol.* 1997;272:H2793-806. doi: 10.1152/ajpheart.1997.272.6.H2793]
34. Bogdanov KY, Vinogradova TM, Lakatta EG. Sinoatrial nodal cell ryanodine receptor and Na^{+} - Ca^{2+} exchanger: molecular partners in pacemaker regulation. *Circ Res.* 2001;88:1254-8.
35. Ju YK, Chu Y, Chaulet H, *et al.* Store-operated Ca^{2+} influx and expression of TRPC genes in mouse sinoatrial node. *Circ Res.* 2007;100:1605-14. doi: 10.1161/CIRCRESAHA.107.152181]

36. Groenke S, Larson ED, Alber S, *et al.* Complete atrial-specific knockout of sodium-calcium exchange eliminates sinoatrial node pacemaker activity. *PLoS One.* 2013;8:e81633. [PMID: 3836769 doi: 10.1371/journal.pone.0081633]
37. Lakatta EG, Maltsev VA, Vinogradova TM. A coupled SYSTEM of intracellular Ca²⁺ clocks and surface membrane voltage clocks controls the timekeeping mechanism of the heart's pacemaker. *Circ Res.* 2010;106:659-73. [PMID: 2837285 doi: 10.1161/CIRCRESAHA.109.206078]
38. Osterrieder W, Noma A, Trautwein W. On the kinetics of the potassium channel activated by acetylcholine in the S-A node of the rabbit heart. *Pflugers Arch.* 1980;386:101-9.
39. Demir SS, Clark JW, Giles WR. Parasympathetic modulation of sinoatrial node pacemaker activity in rabbit heart: a unifying model. *Am J Physiol.* 1999;276:H2221-44. doi: 10.1152/ajpheart.1999.276.6.H2221]
40. Hoffman BF, Cranefield PF. *Electrophysiology of the heart.* New York: McGraw-Hill; 1960.
41. Monfredi O, Dobrzynski H, Mondal T, *et al.* The anatomy and physiology of the sinoatrial node--a contemporary review. *Pacing Clin Electrophysiol.* 2010;33:1392-406. doi: 10.1111/j.1540-8159.2010.02838.x]
42. Boyett MR, Inada S, Yoo S, *et al.* Connexins in the sinoatrial and atrioventricular nodes. *Adv Cardiol.* 2006;42:175-97. doi: 10.1159/000092569]
43. Wu J, Schuessler RB, Rodefeld MD, *et al.* Morphological and membrane characteristics of spider and spindle cells isolated from rabbit sinus node. *Am J Physiol Heart Circ Physiol.* 2001;280:H1232-40. doi: 10.1152/ajpheart.2001.280.3.H1232]

44. Kwong KF, Schuessler RB, Green KG, *et al.* Differential expression of gap junction proteins in the canine sinus node. *Circ Res.* 1998;82:604-12. doi: 10.1161/01.res.82.5.604]
45. Mezzano V, Liang Y, Wright AT, *et al.* Desmosomal junctions are necessary for adult sinus node function. *Cardiovasc Res.* 2016;111:274-86. [PMID: 4957488 doi: 10.1093/cvr/cvw083]
46. Boineau JP, Canavan TE, Schuessler RB, *et al.* Demonstration of a widely distributed atrial pacemaker complex in the human heart. *Circulation.* 1988;77:1221-37. doi: 10.1161/01.cir.77.6.1221]
47. Schuessler RB, Boineau JP, Saffitz JE, *et al.* Cellular mechanisms of Sinoatrial Activity. In: Zipes DP, Jalife J, editors. *Cardiac Electrophysiology from Cell to Bedside.* Philadelphia, London, Toronto, Montreal, Sidney, Tokio: W. B. Saunders Company; 2000. p. 187-95.
48. Verheijck EE, Wessels A, van Ginneken ACG. Morphological diversity of isolated nodal cells from the rabbit SA node does not have functional significance. In: Verheijck EE, editor. *Pacemaker currents in the sino-atrial Node.* Amsterdam: Drukkerij Bevrijding; 1994. p. 11-30.
49. Sanchez-Quintana D, Cabrera JA, Farre J, *et al.* Sinus node revisited in the era of electroanatomical mapping and catheter ablation. *Heart.* 2005;91:189-94. [PMID: 1768731 doi: 10.1136/hrt.2003.031542]
50. Boyett MR, Honjo H, Kodama I. The sinoatrial node, a heterogeneous pacemaker structure. *Cardiovasc Res.* 2000;47:658-87. doi: 10.1016/s0008-6363(00)00135-8]

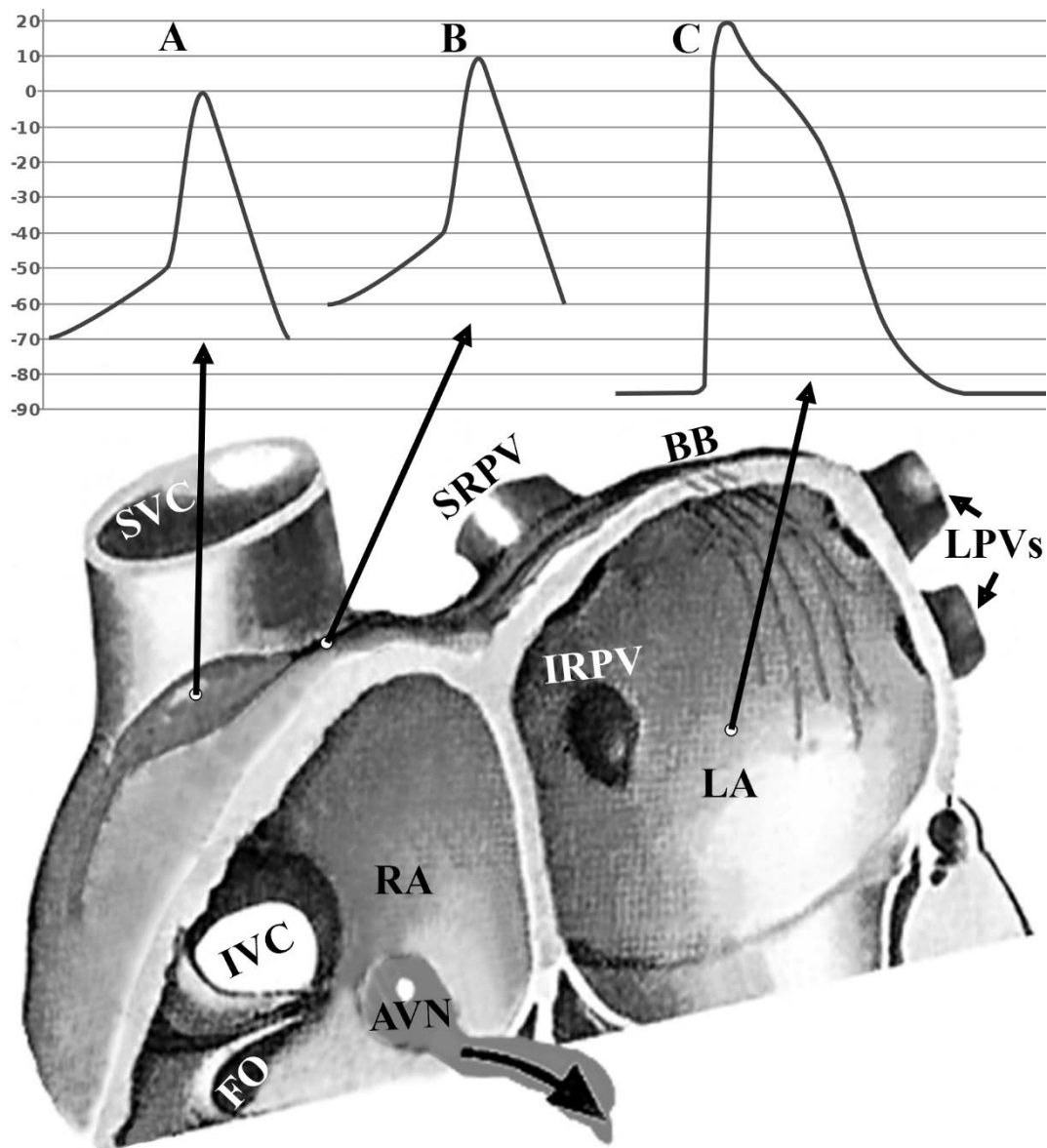
Figure legends

Figure 1 SAN Action Potential



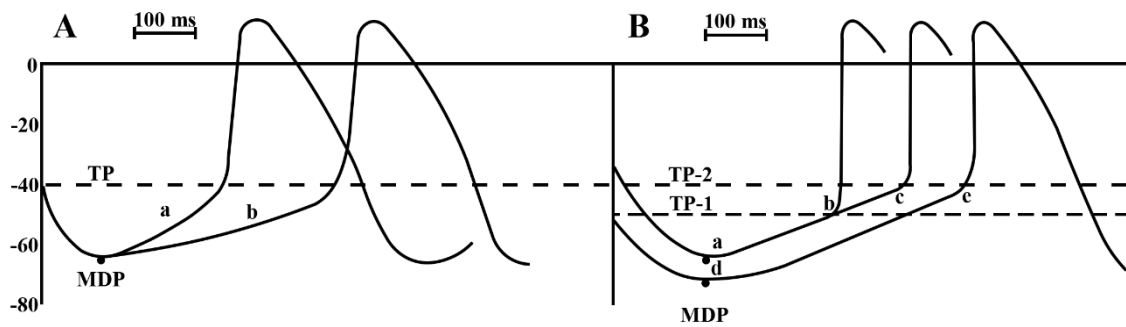
a: Funny current is located mainly in pacemaker cells, these channels become active at very negative membrane potentials (≈ -60 mV) and allow for the passage of both Na^+ and K^+ into the cell; **b:** Transient or T-type Ca^{2+} channel and are found mainly within atrial and pacemaker cells, but still to a lesser degree than L-type channels. **c:** Long-lasting L-type Ca^{2+} current or L-type Ca^{2+} channels are more common and are most densely populated within the t-tubule membrane of ventricular cells; **d:** Phase 3 potassium (K^+) voltage-gated delayed potassium repolarization operate channels; **MDM:** Maximal Diastolic Potential; **TP:** Threshold Potential. **4:** Phase 4 of Action Potential; **0:** Phase 0 L-type Ca^{2+} current (primarily) and later T-type Ca^{2+} channel; **3:** voltage-gated delayed repolarization K^+ channels.

Figure 2 Action potentials at the center, at the end of SAN and atrial tissue



Samples of APs recorded from different regions of the SAN, from the center (region of central SAN) (A) to the periphery of the SAN (lowest black point outside blue region, in SAN periphery) (B), a schematic drawing of the right atrium with the SAN region (C). The corresponding APs from each area with their maximal diastolic potential, and threshold potential, are shown on the up of the figure. AVN: atrioventricular node; BB: Bachman's bundle; FO: fossa ovalis; IRPV: inferior right pulmonary vein; IVC: inferior vena cava; LA: left atrium; LPVs: left pulmonary veins; RA: right atrium; SRPV: superior right pulmonary vein; SVC: superior vena cava.

Figure 3 Mechanisms of modulation of cardiac pacemaker rate



Panel A: two SAN-APs are illustrated. The threshold potential (TP) for both APs is -40 mV. The Maximal Diastolic Potential (MDP) is approximately -60 mV. If the slope of spontaneous depolarization decreases (b), the pacemaker firing rate is delayed. Panel B: three SAN-APs are shown. A decrease in the TP (i.e., less negative, TP-1 to TP-2) results in a delay in firing rate (c versus b). Pacemaker firing rate can also be delayed (e versus c) by an increase in the MDP (i.e., a more negative diastolic potential) (a versus d), with the slope of spontaneous depolarization and the TP remaining constant [40].