The sinoatrial node (SAN) initiates the heartbeat and controls rate and rhythm of contraction. Extensive work in small mammals provided important information about the ionic mechanisms responsible for the generation of the unique action potential shape of the SAN and its spontaneous pacemaker activity. Despite great progress in understanding SAN function, the mechanisms underlying pacemaking in humans are incompletely understood.

The SAN is specifically equipped for its role as the primary pacemaker. The rhythmic action potentials are preceded by slow diastolic depolarization (DD), which brings the membrane potential to the threshold needed for excitation. Extensive connective tissue insulates the SAN from the right atrium and prevents interference of the negative atrial (resting) membrane potential with pacemaking. DD and SAN automaticity are driven by a complex interaction between different voltage-gated ion currents exclusively residing in the surface membrane of the SAN cells (membrane clock hypothesis). According to this hypothesis, the kinetics of channel activation and inactivation control the timing mechanisms of the currents that comprise the membrane clock.

The slope of early DD is determined by the hyperpolarization-activated cyclic nucleotide-gated inward current (I\textsubscript{f}) and, to a lesser extent, low-voltage-activated T-type Ca\textsuperscript{2+} currents (I\textsubscript{Ca,T}). Mutations in the pore-forming Na\textsubscript{1.5} sodium channel subunit have been linked to bradycardia in humans. However, cardiac sodium channels are expressed in the peripheral region of the SAN only and their precise role in pacemaking is not fully understood.

The membrane clock hypothesis of pacemaking is well established, but recent findings by Dr Lakatta and colleagues have challenged the dominant role of the membrane clock mechanism. They provided evidence that local subsarcolemmal Ca\textsuperscript{2+} releases from the sarcoplasmic reticulum (SR) during late DD activate the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger (NCX1) to generate depolarizing exchanger current bringing the late pacemaker potential to the activation threshold of propagated action potential. The local subsarcolemmal SR Ca\textsuperscript{2+} releases occur either spontaneously or in response to I\textsubscript{Ca,T} activation. Thus, in SAN cells, SR can spontaneously cycle Ca\textsuperscript{2+}, and these rhythmic SR Ca\textsuperscript{2+} releases were proven critical contributors to the initiation of pacemaking.

Interestingly the Ca\textsuperscript{2+} clock ticks shortly before the subsequent action potential, which intuitively indicates that the Ca\textsuperscript{2+} clock may be the driver of pacemaking. In small mammals, there is evidence for both pacemaking mechanisms. The molecular basis of pacemaking in the human heart, however, is less well understood. Blockade of I\textsubscript{f} current with ivabradine produces bradycardia, and mutations of the major I\textsubscript{f}-channel subunit HCN4 are associated with sick sinus syndrome in affected families, suggesting involvement of the membrane clock mechanism in pacemaking in humans. However, whether the Ca\textsuperscript{2+} clock mechanism also contributes to pacemaking is unknown.

Given the great efforts made to develop biological pacemakers for treatment of sick sinus syndrome or disease-related SAN dysfunction and the limited knowledge of the precise molecular mechanisms of pacemaking in humans, studies specifically addressing human SAN function and dysfunction are of physiological and clinical importance.

In this issue of Circulation, Chandler et al provide important insights into the expression profiles of ion channels, Ca\textsuperscript{2+}-handling proteins, connexins, and membrane receptors in the human SAN. Specifically, they carefully dissected the specific differences in ion channel signatures between SAN and right atrium and identified a novel, anatomically-separated SAN-like region (paranodal [PN] area) in right atrium that showed an intermediate expression phenotype. Integrating the mRNA abundance of ion channel subunits as an index of ion current conductance, the authors modeled the human SAN action potential to predict the functional consequences of their findings. Surprisingly, the inclusion of the SAN-specific expression profiles of only 9 ion currents (I\textsubscript{Na}, I\textsubscript{Ca,L}, I\textsubscript{Ca,T}, I\textsubscript{f}, and 5 key K\textsuperscript{+} currents) and 3 Ca\textsuperscript{2+}-handling processes (SR Ca\textsuperscript{2+} release through ryanodine channels [RyR2], Ca\textsuperscript{2+} uptake via SR Ca\textsuperscript{2+}-ATPase2a [Serca2a] and Ca\textsuperscript{2+} extrusion through NCX1) into the model was sufficient to generate a typical SAN cell action potential.

The authors concluded that the complex pattern of ion channel expression in human SAN is sufficient to explain pacemaking. They emphasized that the molecular architecture of the human SAN needs critical consideration for the design of biological pacemakers.
The study by Chandler et al\textsuperscript{7} provides the first comprehensive characterization of the molecular make-up of the human SAN but raises critical questions that warrant direct experimental verification in subsequent work. In principle, the authors confirmed abundance of typical SAN genes (HCN4, Ca.1.3 contributing to I\textsubscript{Ca,L}, Ca.3.1 underlying I\textsubscript{Ca,T}) and the characteristic absence (or reduced expression) of atrial muscle genes (Na.1.5, connexin43, atrial natriuretic peptide) in the human SAN. The mRNA levels of Kir2.1 and Kir2.3, which constitute I\textsubscript{K,I} as the major determinant of stable resting membrane potential, are much lower in the SAN than in the right atrium. In analogy to rabbit SAN, the small (or absent) I\textsubscript{K,I} current may be the reason for the more positive resting membrane potential in human SAN cells.\textsuperscript{8} Indirectly, this supports pacemaking. The HCN4 mRNA abundance was much lower in human compared with rabbit SAN,\textsuperscript{4} and this is in good agreement with the 3 to 4 times smaller If amplitude of the Ca\textsuperscript{2+}ing. Although this does not necessarily exclude a contribution to pacemaking.

The Ca\textsuperscript{2+} handling proteins but still reproduced the expected AP shape. For instance, normal ion current activity requires accessory ICa,L–mediated Ca\textsuperscript{2+} release with subsequent NCX1 current activation. On the basis of this dynamic interaction between the 2 pacemaking mechanisms, the membrane clock was thought to be insufficient to sustain pacemaking in the absence of the Ca\textsuperscript{2+} clock.\textsuperscript{6} It is notable that the mathematical model used by Chandler et al\textsuperscript{7} totally ignored key aspects in the regulation of ion currents and Ca\textsuperscript{2+} handling proteins but still reproduced the expected AP shape. For instance, normal ion current activity requires accessory subunits for fine-tuning of proper function. Also, the rhythmic subsarcolemmal Ca\textsuperscript{2+} releases underlying the Ca\textsuperscript{2+} clock mechanism depend on higher protein kinase A phosphorylation of Serca2a-inhibitory phospholamban and RyR2 in the SAN.\textsuperscript{10} Despite these limitations, the model is consistent with the membrane clock hypothesis and shows that the reduction of I\textsubscript{K,I} current results in slowing of pacemaking. In the configuration of the model, however, the Ca\textsuperscript{2+} clock seems less important for pacemaking, because removal of the 3 key Ca\textsuperscript{2+}-handling processes did not significantly affect pacemaking. Although this does not necessarily exclude a contribution of the Ca\textsuperscript{2+} clock to pacemaking, it at least questions the dominance of this mechanism for pacemaking in humans. This issue can only be resolved with direct measurements in human SAN cells.

The SANs of rabbits have region-specific differences in cell morphology and function.\textsuperscript{4} Cells located in the central SAN area, the leading pacemaking site, have longer action potentials, slow upstroke velocity, small amplitude, and a more positive resting membrane potential. In contrast, peripheral SAN cells have stronger electrical coupling, shorter action potentials, faster upstroke velocity, larger amplitude, and a more negative resting membrane potential. In addition, expression of Ca\textsuperscript{2+}-handling proteins (NCX1, RYR2, and Serca2a) increases from the center to the periphery of the rabbit SAN, suggesting that the Ca\textsuperscript{2+} clock mechanism of pacemaking may vary between the 2 regions.\textsuperscript{1} The SAN periphery obviously insulates the central part from the atrial myocardium but also facilitates impulse exit and propagation to atrial tissue. The region-specific differences in cell function are beyond the scope of the study of Chandler et al\textsuperscript{7}; further studies, however, are clearly warranted to settle this issue.

The regulating mechanisms of the unique ion-channel expression profile in the SAN are not addressed in the study by Chandler et al.\textsuperscript{7} The T-box transcription factor Tbx3, a transcriptional repressor required for development in vertebrates, was recently identified as a critical regulator of the pacemaker gene expression program and phenotype.\textsuperscript{11} Tbx3 was present in human SANs but not in the right atrium, indicating its potential involvement in regulating human SANs.

The study by Chandler et al\textsuperscript{7} identified a novel PN area that showed not only some similarities to SAN expression profile but also differences in the ion channel transcripts repertoire compared with the right atrium. As noted by the authors, the K\textsuperscript{+} channel make-up of the PN area suggests that these cells may be depolarized compared with right atrial myocytes, thereby facilitating the conduction of the action potential from the SAN into the right atrium. In addition, this area may play a role in shifting the location of the leading pacemaker site in response to sympathetic and parasympathetic stimulation.\textsuperscript{1} Although the physiological role of the PN area remains to be determined, the presence of SAN-like cells outside of the SAN may be important for atrial arrhythmogenesis. In fact, ectopic sites along the crista terminalis are known sources of atrial tachycardias, and ablation of these sites terminates the arrhythmias.\textsuperscript{12}

Age, heart failure, and atrial fibrillation (AF) are frequent causes of SAN dysfunction with intrinsic decrease in heart rate.\textsuperscript{1} AF-related atrial remodeling is an important determinant of atrial arrhythmogenesis.\textsuperscript{13} Rapid atrial pacing in dogs produces atrial tachycardia remodeling in both the atrium and in the SAN,\textsuperscript{14} and patients with atrial flutter or AF often develop bradyarrhythmia-tachycardia syndrome.\textsuperscript{15} However, specific information about the distinct components of AF-related SAN remodeling in humans is lacking. In this sense, the dissection of the molecular SAN make-up by Chandler et al\textsuperscript{7} is an important step in the exploration of the arrhythmia-related SAN ion channel remodeling. Further studies in experimental AF models and in atrial tissue from AF patients are needed to delineate the arrhythmia-associated SAN alterations and their underlying mechanism(s).

In summary, Chandler et al\textsuperscript{7} performed a comprehensive analysis of the molecular architecture of the healthy human SAN and provided novel mechanistic insights into the molecular basis of pacemaking in the human heart. These results have important implications for understanding disease-related SAN remodeling and for predicting the results of therapeutic interventions. Appreciation of SAN-specific ion channel make-up in humans may provide new opportunities for
treatment of SAN dysfunction associated with sick sinus syndrome, heart failure, or AF.

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None.

References

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