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How Does Cholinergic Activation Slow Down Sinus Node Automaticity? "Diastolic Voltage Oscillations" vs. "Calcium Clock" Mechanisms

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Editorial Comment

Claudius Galen (129–199 AD)was perhaps the first to recognize the intrinsic ability of the heart to beat spontaneously. He was perplexed to see the excised heart of animals continue beating spontaneously. In his words: "The power of pulsation has its origin in the heart itself…. The fact that the heart, removed from the thorax, can be seen to move for a considerable time is a definite indication that it does not need the nerves to perform its own function."[1] Unfortunately, this remarkable observation remained a mystery and unexplained for a very long time. Even William Harvey, in his monumental "De motu cordis" published in 1628, does not address the question of the origin of the heartbeat. It was not until centuries later that the structure and function of what "moved the heart" was identified and studied in detail. The pioneering discovery by Walter Gaskell in 1886 that the sinus venosus in the heart was the site of the first excitation of the heart was a turning point. Working with an isolated strip of tortoise ventricular muscle, Gaskell showed that the strip continued to pulsate at a rate similar to that of the intact heart and demonstrated in the slowly beating tortoise heart that the propagation of the heartbeat proceeded as a peristaltic wave from the sinus venosus to the nearby atrium.[1,2] Equally interesting in Gaskell's discovery was the demonstration of the presence of what is known today as the "dominant pacemaker" in the sinus venosus: "The heart beat starts from that part which is most rhythmical, i.e., the part which beats spontaneously at the quickest rate, and travels as a wave of contraction over the rest of the heart…. "[2] The first histological description of "what moved the heart" in animals and humans was provided by Keith and Flack in 1907, who dubbed it "the sinu-auricular node" (SAN).[3] In 1910–1911, with the availability of methods capable of detecting and recording electrical activity in cardiac tissue, Sir Thomas Lewis and associates demonstrated the SAN to be the site of the mammalian pacemaker.[4]

Although the myogenic origin of the heartbeat gained early momentum, there were parallel investigations that recognized the profound influence exerted by the activation of the autonomic nervous system in modulating the heart rate. For example, as early as in 1812, the

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French physiologist Cesar Legallois theorized on the basis of his crushed spinal cord animal experiments that the heart rate was under nervous control.[1] Subsequent discovery in mid 19th century of the sympathetic and parasympathetic nerves and ganglia inside and outside the heart, and the experimental demonstration of heart rate changes by direct electrical (galvanic) stimulation of the nerves provided compelling evidence of neural control of the heart rate. One of the important discoveries in the 19th century was the demonstration of the negative chronotropic effect by electrical stimulation of the vagus nerve by Eduard and Ernst Weber in 1845.[1] Seventy-five years later, the brilliant experiments of the German pharmacologist Otto Loewi in 1921 provided the first experimental proof of chemical mediation of heart rate slowing by the vagus nerve.[5] Loewi stimulated the vagus nerve of a perfused frog heart (donor) and allowed the perfusion fluid to come in contact with a second frog heart (recipient), resulting in slowing of heart rate in the recipient as well. Loewi deduced that a substance must have been liberated by the donor heart that slowed the rate of the recipient heart. He referred to this substance as Vagusstoff (vagus substance),[6] which in 1926 was identified to be acetyl choline (Ach) by Loewi and Navaratti.[6] Loewi's discovery was eventually confirmed in warm blooded animals with more highly organized nervous systems as Rylant in 1927 extended Loewi's frog experiments to rabbits[7] and Feldberg and Krayer in 1933 to cats and dogs.[8]

The last 40 years witnessed a remarkable increase in basic research designed to elucidate the ionic basis of sinus nodal cell pacemaker activity. These studies brought forth 2 fundamentally different mechanisms of generation of the characteristic U-shaped phase 4 spontaneous diastolic depolarization (SDD): the "membrane voltage clock" and the "Ca clock" mechanisms. Initially perceived as exclusionary, these 2 mechanisms were later found arguably to be somewhat complementary[9,10] and even synergistic.[11]

The "membrane voltage clock" or sarcolemmal ionic mechanism relies on an inward depolarizing ("funny") current (I_f) that becomes activated upon repolarization of the SAN cell causing phase 4 SDD and firing of an action potential (AP).[12,13] A limitation of this mechanism is that the I_f is too small in the dominant pacemaker cells that have a maximum diastolic potential (MDP) of < −60 mV, a voltage close to the I_f reversal potential (~ −40 mV). This raised doubts that I_f could be the exclusive pacemaker current of the SAN cells.[13] Soon thereafter, the "Ca clock" mechanism emerged as an important component of the pacemaker current. The postulation was made possible with the advent of technology that measures changes in the intracellular calcium ion concentrations ($[Ca_i^{2+}]$) in isolated pacemaker cells using Ca-sensitive fluorescent dyes, laser scanning, and confocal imaging.[9,14] It is postulated that the SAN automaticity arises from "spontaneous" and "critically timed" (during the late diastole) local subsarcolemmal Ca^{2+} releases via the ryanodine receptors of the sarcoplasmic reticulum (SR). The Ca release activates sarcolemmal Na–Ca exchanger current in the forward direction (extruding 1 Ca ion for the inflow of 3 Na ions) generating a net inward depolarizing current causing phase 4 SDD. Accordingly, the period of sinus automaticity becomes regulated by the period of spontaneous Ca release. One release per one beat and the faster the release, the faster the rate. The presence of positive coupling between the dynamics of the SR Ca^{2+} cycling and

sinus node automaticity (the higher the Ca_i^{2+}, the greater the pacemaker current) is taken as further supportive evidence for the Ca clock mechanism.[9]

In this issue of the *Journal*, Vassalle and associates revisit the theme of cholinergic slowing of heart rate, and assert that the mechanism still remains mired with "controversy" some 90 years after its discovery.[15] The study by these authors is based on a method that they developed more than a decade ago. The method relies first on "unmasking" the fine structure of the U-shaped phase 4 SDD and then testing how cholinergic activation affects these diastolic oscillations to slow or arrest sinus automaticity.[15,16] Additionally, these studies rely on observations of diastolic voltage processes during the recovery of sinus node automaticity after removing cholinergic sinus arrest. This analytical method, which remained outside the purview of the 2 major proposed mechanisms of automaticity, uses isolated-superfused guinea pig sinus nodal tissue, from which simultaneous recordings of single cell APs with glass microelectrode and force of contraction (as a surrogate for Ca_i^{2+} loading) are made. The basic tenet of this approach is to separate ("unmask") the "fused" fine structure of the U-shaped SDD into its constituting components by progressively raising the extracellular K concentrations ($[K^+]_0$) in the range of 10–14 mM. Raising $[K^+]_0$ also converts the subsidiary pacemakers into the dominant form of pacemaker (MDP less negative than −65 mV) so the SAN tissue becomes populated by the dominant form of the pacemaker cells. This is shown by recordings of the U-shaped smooth phase 4 SDD characteristics of dominant pacemaker cells. With this method, the authors show that the U-shaped SDD becomes separated into (1) an early DD, (2) oscillatory afterpotentials that follow the DD and termed as Vos (voltage oscillations), and (3) threshold prepotentials (ThVos) that follow the Vos and just precede the sinus discharge.[17] The authors assert that the DD, Vos, and ThVos are all fused together under normokalemic conditions manifesting the characteristic U-shaped phase 4 SDD, but become separated from each other when $[K^+]_0$ is elevated. Since progressive recovery from high to normal $[K^+]_0$ leads to progressive fusing of the DD, Vos, and ThVos into a single U-shaped SDD, the authors conclude that the U-shaped spontaneous depolarization results from the summation of the DD, Vos, and ThVos. With this approach, Vassalle and associates find that the slowing of the sinus rate by low levels of carbachol (CCh) is associated with a decrease in the "amplitude and the slope" of the Vos, ThVos with "no change in the period" of the oscillatory diastolic potentials. Based on this and absent CCh effect on sinus node AP shape or MDP, these authors conclude that the CCh slowing is not caused by "changing SAN discharge through changes in its rate as a 'clock' would do." Instead, it is suggested to be caused by the lowering of the amplitude and the slope of diastolic oscillatory depolarizations intermittently missing the threshold leading to slowing of the rate.[15]

Are Vos and ThVos a continuum? The authors argue to the contrary. They assert that the Vos are "afterpotentials," like EADs and DADs (i.e., triggered activity), and therefore require a prior AP to produce them. In contrast, the ThVos are treated as "prepotentials" and their spontaneous emergence during recovery from CCh-induced sinus arrest is argued to disqualify them as "afterpotentials" due to the lack of a prior AP to produce them. The ionic mechanisms of these oscillatory potentials were not determined in this study. The authors suggest that cellular Vos and ThVos may be related to diastolic Ca release by the SR. While

it is possible that ThVos ("prepotential") may result secondary to spontaneous Ca release from the overloaded SR after a long period of quiescence, the same could not be said about Vos, which immediately follows the DD. Another puzzling aspect of this study is the nature and the role of the early "DD1" that immediately follows repolarization. The authors provide no ionic information of its nature and its role in cholinergic slowing of SAN automaticity. While the authors show that the DD1 is insensitive to CCh and to the I_f blocker cesium chloride (2 mM), its potential role in automaticity, however, remains unexplored in this study. An early study by West is compatible with the oscillatory diastolic potential hypothesis of Vassalle and associates. By progressively decreasing the extracellular Na ion concentration ($[Na^+]_0$, West showed that the slowing of sinus node automaticity was associated with the emergence of subthreshold diastolic oscillatory potentials with reduced amplitudes and slopes, but with a period that is similar to control sinus node automaticity.[4] Low $[Na^+]_0$ and high $[K^+]_0$ appear to unmask diastolic oscillatory potentials with a similar period as that of the control, albeit with lower amplitude and slopes. Diastolic oscillatory mechanism argues against the timed Ca release mechanism of CCh-induced slowing of sinus automaticity. Conceptually, it would seem that the SAN cells have an intrinsic ability to produce diastolic oscillatory potentials with a set period, a concept that needs to be proven. According to Vassalle and associates, the chronotropic agents would change the sinus rate by changing the amplitude and the slope of the oscillatory diastolic potentials, not by changing the period of the oscillations. While the authors dismiss the "Ca clock mechanism" as a cause of sinus slowing, they do attribute a considerable importance to Ca_i^{2+} dynamics in SAN automaticity. They not only observe that the slowing of the sinus rate is associated with a concomitant decrease in the force of contraction (twitch) (Ca unloading), but they also show that low extracellular concentrations of Ca^{2+} mimic the slowing effect of CCh. Furthermore, rapid pacing (Ca loading) transiently reverses CCh-induced slowing consistent with calcium load exerting positive chronotropic influence on sinus discharge. This point appears consistent with an earlier finding by these authors showing suppression of Vos by ryanodine.[17] This dynamic scenario is consistent with elevated $[Ca_i^{2+}]$ stimulating the extrusion of calcium by the Na^+–Ca^{2+} exchanger generating an inward current "superimposed on DD1."[18] Finally, the authors also dismiss any role for the I_f in pacemaking based on the inability of I_f block with 2 mM cesium chloride to change the profile of CCh-mediated slowing of the SAN automaticity. At higher concentrations, CCh hyperpolarizes the cell shifting the membrane potential away from the "oscillatory zone," consistent with CCh-induced activation of muscarinic K channels ($I_{K,ACh}$). Here again the authors dismiss any role for I_f in sinus slowing with high CCh by showing that reduction in the amplitude and the slope of Vos, ThVos, and force of contraction precede hyperpolarization with high CCh.

It remains a challenge to ascertain if the U-shaped SDD truly reflects the summation of different diastolic oscillatory processes. Future studies need to confirm and extend the present tissue level findings to isolated sinus nodal cell level. With the use of confocal microscopy and patch clamp technique to measure the I_f and Na–Ca exchanger current, insight could be obtained on the presence and the role of these elusive oscillatory diastolic potentials in isolated SAN cells. To the extent that summation of the oscillatory diastolic potentials observed with high $[K^+]_0$ at the tissue level reflect the characteristic U-shaped

phase 4 SDD at single cell level, these findings may provide novelmechanistic insight into the mechanisms of SAN automaticity that may also be useful in the therapy of sinus node dysfunction.

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References

1. Fye WB. The origin of the heart beat: A tale of frogs, jellyfish, and turtles. Circulation. 1987; 76:493–500. [PubMed: 3304703]
2. Silverman ME, Grove D, Upshaw CB Jr. Why does the heart beat? The discovery of the electrical system of the heart. Circulation. 2006; 113:2775–2781. [PubMed: 16769927]
3. Keith A, Flack M. The form and nature of the muscular connections between the primary divisions of the vetebrate heart. J Anat Physiol. 1907; 41:172–189. [PubMed: 17232727]
4. West, TC. Electrophysiology of the sinus node. In: De Mello, WC., editor. Electrical Phenomena in the Heart. New York & London: Academic Press; 1972. p. 191-217.
5. Loewi O. Über humorale Übertragbarkeit der Herznervenwirkung. Pflügers Arch ges Physiol. 1921; 189:239–242.
6. Loewi O, Navratil E. Über humorale Übertragbarkeit der Herznervenwirkung. X. Mitteilnung. Über das Schicksal des Vagusstoffs. Pflügers Arch ges Physiol. 1926; 214:678–688.
7. Ryland P. La “transmission humorale de l’action des nerfs cardiaques” de Loewi chez la mammifère. C. R. Sénac Soc Biol. 1927; 96:204–205.
8. Feldberg W, Krayer O. Das Auft eines azetylcholinartigen Stoffes in Herzvnenenblut von Warmblütern bei Beizung der Nervi vagi. Arch. exp.Path. Pharmak. 1933; 172:170–193.
9. Lakatta EG, DiFrancesco D. What keeps us ticking: A funny current, a calcium clock, or both? J Mol Cell Cardiol. 2009; 47:157–170. [PubMed: 19361514]
10. Ju YK, Chu Y, Chaulet H, Lai D, Gervasio OL, Graham RM, Cannell MB, Allen DG. Store-operated Ca^{2+} influx and expression of TRPC genes in mouse sinoatrial node. Circ Res. 2007; 100:1605–1614. [PubMed: 17478725]
11. Joung B, Tang L, Maruyama M, Han S, Chen Z, Stucky M, Jones LR, Fishbein MC, Weiss JN, Chen PS, Lin SF. Intracellular calcium dynamics and acceleration of sinus rhythm by beta-adrenergic stimulation. Circulation. 2009; 119:788–796. [PubMed: 19188501]
12. DiFrancesco D, Ferroni A, Mazzanti M, Tromba C. Properties of the hyperpolarizing-activated current (if) in cells isolated from the rabbit sino-atrial node. J Physiol. 1986; 377:61–88. [PubMed: 2432247]
13. DiFrancesco D. The role of the funny current in pacemaker activity. Circ Res. 2010; 106:434–446. [PubMed: 20167941]
14. 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. [PubMed: 11420301]
15. Vassalle M, Nett MP, Catanzaro JN, Rota M. Novel oscillatory mechanisms in the cholinergic control of guinea pig sino-atrial node discharge. J Cardiovasc Electrophysiol. 2011; 22:71–80. [PubMed: 20662981]
16. Gonzalez MD, Vassalle M. Role of oscillatory potential and pacemaker shifts in digitalis intoxication of the sinoatrial node. Circulation. 1993; 87:1705–1714. [PubMed: 8387899]
17. Nett MP, Vassalle M. Obligatory role of diastolic voltage oscillations in sino-atrial node discharge. J Mol Cell Cardiol. 2003; 35:1257–1276. [PubMed: 14519436]
18. Vassalle M, Lin CI. Calcium overload and cardiac function. J Biomed Sci. 2004; 11:542–565. [PubMed: 15316129]