Reviews

This Review is part of a thematic series on **Mechanisms of Pacemaking in the Heart**, which includes the following articles:

- Development of the Pacemaker Tissues of the Heart *[Circ Res. 2010;106:240–254]*
- The Role of the Funny Current in Pacemaker Activity *[Circ Res. 2010;106:434–446]*
- A Coupled SYSTEM of Intracellular Ca²⁺ Clocks and Surface Membrane Voltage Clocks Controls the Timekeeping Mechanism of the Heart’s Pacemaker *[Circ Res. 2010;106:659–673]*

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**Competing Oscillators in Cardiac Pacemaking: Historical Background**

Denis Noble, Guest Editor, and Brian O’Rourke, Editor

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**Competing Oscillators in Cardiac Pacemaking**

**Historical Background**

Denis Noble, Penelope J. Noble, Martin Fink

**Abstract:** Interaction between a membrane oscillator generated by voltage-dependent ion channels and an intracellular calcium signal oscillator was present in the earliest models (1984 to 1985) using representations of the sarcoplasmic reticulum. Oscillatory release of calcium is inherent in the calcium-induced calcium release process. Those historical results fully support the synthesis proposed in the articles in this review series. The oscillator mechanisms do not primarily compete with each; they entrain each other. However, there is some asymmetry: the membrane oscillator can continue indefinitely in the absence of the calcium oscillator. The reverse seems to be true only in pathological conditions. Studies from tissue-level work and on the development of the heart also provide valuable insights into the integrative action of the cardiac pacemaker. *(Circ Res. 2010;106:1791-1797.)*

**Key Words:** cardiac pacemaker ■ heart models ■ calcium oscillator ■ membrane oscillator

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The earliest models of cardiac rhythm, beginning with that of Noble, were restricted to interactions between surface membrane ion channels. The first cardiac cell model to incorporate the intracellular calcium signaling system involved in excitation–contraction coupling was that of DiFrancesco and Noble. That model was developed for sheep Purkinje fibers, and it was the first to predict a large role for sodium/calcium exchange current during the action potential. In fact it identified the slower components of inward current that had previously been attributed to calcium channel current as being attributable instead to the exchanger. Pacemaker activity in that model was attributed almost entirely to the slow onset of the nonspecific cation current, $i_\mathrm{f}$, activated by hyperpolarization. The exchange current was not predicted to play any role in pacemaker rhythm in that case.

However, the DiFrancesco–Noble Purkinje fiber model was almost immediately developed to create the first mathematical model to reproduce voltage waveforms similar to those observed in rabbit sinoatrial node (SAN) cells. It was also developed later to create the first models of rabbit atrial cells. For the intracellular calcium signaling mechanisms, the 1980s models were based on the work of Fabiato on calcium-induced calcium release.

The experimental basis of the SAN model was the work of Brown et al, which revealed the presence of very slow components of inward current similar to that predicted for the
sodium/calcium exchange current during the action potential. In fact, the modeling gave confidence that these experimental recordings were not voltage-clamp artifacts.

One of the collaborators in that experimental work, Junko Kimura, later collaborated with Akinori Noma to measure the voltage and ion concentration dependence of the sodium/calcium exchange current under highly controlled conditions. The results were in remarkable agreement with the equations used in the models. All subsequent developments of models of calcium signaling and of sodium/calcium exchange in the heart can be seen to derive from the models of the 1980s.

Among the experimental results on the rabbit SAN, there were 2 important findings that relate to the present controversies. The first was that, when investigating inward currents during voltage-clamp depolarizations, 2 clear components were often observed. Based on the modeling work, the first of these was attributed to activation of the L-type calcium current (called \( I_{Na} \) in early work), whereas the second, much slower, component was attributed to activation of sodium/calcium exchange during calcium release following the onset of the action potential.

The computer model correctly reproduced these 2 components (see Figure 9 in Brown et al.). Those original computations were done using OXSOFT HEART. We have repeated the computations for this article using the publicly available software COR (www.cor.physiol.ox.ac.uk) and the CellML encoded version of the model downloaded from the CellML model repository (www.cellml.org). The results are shown in Figure 1. They are very similar to the original figure but extend it by revealing the separate contributions of \( I_{CaL} \) and \( I_{NaCa} \). Later experimental work confirmed the predictions of the models regarding sodium/calcium exchange current during the action potential.

Having established the performance of those models so far as the action potential is concerned, we now turn to the pacemaker depolarization. This is the critical question. During voltage-clamp depolarizations the sodium/calcium exchange current, reflecting the time course of the intracellular calcium transient invariably followed the onset and inactivation of the calcium channel current. By contrast, this time relationship was often reversed during the pacemaker depolarization. Figure 2 shows the experimental protocol used to reveal this in the 1984 work. The natural pacemaker depolarization was interrupted at various times by clamping the voltage to that achieved at the time of onset of the clamp. If this time was late enough (roughly, during the last third of the pacemaker depolarization), a slow inward current was recorded whose time course resembled the slow component recorded during standard voltage-clamp depolarizations. However, there was no visible preceding activation of the calcium current. This was interpreted to indicate the possibility that calcium release during sinus node pacemaker activity could precede activation of the calcium current, as observed by Lakatta et al. Figure 2 shows new computations of this phenomenon using COR and the downloaded CellML file for the 1984 SAN model.

The essence of the Lakatta hypothesis was therefore present in the very earliest simulations of calcium signaling in cardiac cells.

Why did Brown et al not attribute the pacemaker depolarization itself to this mechanism? The main reason was that, although slow inward ionic currents of this kind were often recorded in the experiments, they nearly always died out after 1 or 2 oscillations. The maintenance of the calcium signal oscillator was not therefore independent of the voltage-dependent ionic current oscillator.

**How Do the Different Oscillators Interact?**

To investigate the contribution of the various ion currents, we compared the simulation results of the 6 mathematical models of SAN cells available in the CellML repository: Demir et al., Dokos et al., Kurata et al., Maltsev and Lakatta, Noble and Noble, and Zhang et al. For clarity of the figures, we show only the results of the latest 4 models in the figures but include the results of all models in the text.

Figure 3 shows the membrane potential, the intracellular calcium concentration and the inward currents the models have in common: \( \text{Ca}^{2+} \) L-type current (\( I_{CaL} \)), \( \text{Na}^+/-\text{Ca}^{2+} \) exchanger (\( I_{NaCa} \)), funny current (\( I_f \)), and background sodium current (\( I_{NaB} \)).

The \( I_{CaL} \) shows the largest amplitude of the currents in all the models, and the maximum \( I_{NaCa} \) current is larger than \( I_f \) and \( I_{NaB} \) except in the Zhang model, which has a constant intracellular calcium concentration for model simplification. \( I_f \) is in all models smaller than the \( I_{NaB} \).

When we now block the various currents, one at a time (see Figure 4), what happens to the membrane potential (and calcium) oscillations in the SAN cell models? All the models (Demir, Zhang, Maltsev, Dokos, Noble, Kurata) agree on the importance of \( I_{CaL} \) without activation of this calcium current, the SAN oscillations do not occur. The models are in accord with the experimental observation that without calcium uptake or release from the sarcoplasmic reticulum (SR) the oscillations do not stop (and a 2% increase in background sodium current leads to no missing beats in the Maltsev model). In addition, complete block of \( I_f \) does not stop the oscillations in any of the models.

So, is only \( I_{CaL} \) necessary for the oscillations? What is actually causing the oscillations in the models? In all models, there exist a number of background or sustained inward currents (\( I_{NaB} \), \( I_{Scal} \), \( I_{st} \)), and setting them to zero abolishes or diminishes the oscillations in 4 of the models (Kurata, Maltsev, Noble, Demir); does that mean that the background currents might be more important than \( I_f \) and intracellular \( \text{Ca}^{2+} \) cycling? Clearly, we are dealing here with a multifactorial system in which even ionic currents that show no intrinsic dynamic properties (which is the definition of a “background” current) or currents that have yet to be characterized completely (such as \( I_{st} \)) play an important quantitative role. \( I_{st} \) has been first characterized by Guo et al (1995)
Figure 1. Sodium/calcium exchange currents in the DiFrancesco–Noble (1985) model.2 Top (A), Experimental results obtained by Kimura et al 1987 using guinea pig ventricular cells.10 Exchange current in the inward mode (corresponding to sodium influx and calcium efflux) as a function of membrane potential at different concentrations of extracellular sodium ions. Top (B), Corresponding curves computed from the equations used for sodium/calcium exchange current in the model (note that these results were obtained before the experimental ones). Bottom, Variations of ionic currents ($I_K$, $I_{Ca,L}$ [now $I_{Ca,L}$], $I_f$, and $I_{Na/Ca}$) during computed action and pacemaker potentials. Note the substantial inward exchange current predicted during the plateau of the action potential. This computation was performed for this article using COR and is exactly the same as that published in 1985.
as sustained \(Na^+\) inward current, which is active during the depolarization phase, but because of unavailability of a specific blocker, it has been impossible to investigate the importance of this current in whole SAN cells.

The removal of \(I_{NaCa}\) abolishes the oscillations as well (in all models but the Zhang model which has fixed ion concentrations). \(I_{NaCa}\) is most of the time an inward current extruding \(Ca^{2+}\) from the cell (see Figure 4). When additionally setting

**Figure 2. Calcium release preceding activation of ICaL.** Top left, One of the experimental recordings made on rabbit sinoatrial node by Brown et al (Figure 9 in the article, trace at \(-44\) mV). The membrane potential was allowed to change spontaneously during an action potential and for most of the subsequent pacemaker depolarization. Near the end of this depolarization, but clearly before the upstroke of the action potential, the membrane potential was clamped at the potential reached. A slow transient inward current was recorded, the onset of which is much slower than that of the L-type calcium current. It requires \(-100\) ms to reach its peak. Bottom left, Voltage protocol used to repeat the 1984 simulation of these results using the SAN model developed from the DiFrancesco–Noble model. Vertical scale is in millivolts. Horizontal scale is in seconds. Top right, Computed net ionic currents corresponding to the three levels of the clamp potential in the bottom left protocol. Clamping at the middle of the pacemaker depolarization simply generates a smooth development of net inward current corresponding to decay of \(I_K\) and onset of \(I_f\). The middle curve (interrupted) generates a slow transient inward current similar to that in the experimental trace (top left). The dotted curve generates a double peak as the L-type calcium current starts to be activated. Vertical scale is in nA; horizontal scale in seconds. Bottom right, Computed variations in the sodium/calcium exchange current during the three voltage protocols.

**Figure 3. Overview of models, oscillations, and underlying currents.** Note that \(I_f\) (solid line in bottom graphs) is always the smallest of the inward currents (\(I_{CaL}, I_{NaCa}, I_{Na},\) and \(I_f\)).
the SR Ca$^{2+}$ concentration to constant the block of $I_{\text{NaCa}}$ does only change the frequency, but does not abolish the oscillations completely. Therefore, it is the resulting Ca$^{2+}$ overload with full $I_{\text{NaCa}}$ block that leads to the termination of the oscillations. As there exist also other pumps and exchangers in SAN cells to extrude Ca$^{2+}$ from the cell (avoiding Ca$^{2+}$ overload), which are not present in the mathematical models it is unclear whether complete block of $I_{\text{NaCa}}$ would abolish the oscillations in real cells.

Sensitivity analysis of the SAN period with respect to block of ion currents and pumps was performed at steady state of the model (300 seconds after the change). The Table shows that the models consistently show an increase in period of 0.7 to 1.87%, 0.77 to 4.82%, 0.14 to 0.83%, and 0.83 to 1.34% with a 10% block of $I_{\text{CaT}}, I_{\text{Na}}, I_{\text{f}},$ and $I_{\text{st}},$ respectively. Currents and pumps related to the intracellular calcium system show rather diverse results for the different models (block of $I_{\text{CaT}}, I_{\text{NaCa}}$ and $I_{\text{up}}$ led to a decrease in period in Demir versus and increase in Maltsev). We assume that this lack of consistency is attributable to the differences in calcium handling in the models.

In general, the largest sensitivities can be observed for $I_{\text{BNa}}/I_{\text{st}}$ and second comes $I_{\text{CaT}}$ (ignoring the inconsistent values for $I_{\text{Cat}}$). The Maltsev model provides an exception, with $I_{\text{up}}$ inducing the largest change in period (followed by $I_{\text{BNa}}$ and $I_{\text{CaT}}$).

A bifurcation analysis would be able to provide further information on the dynamic behavior of the models but would be beyond the scope of this article. Note also that all detailed results have to be interpreted with caution because the models have not been tuned and built for these kind of investigations, i.e., the analysis could be out of the predictive range of the models.

The models would suggest 3 important features of concerted action leading to and maintaining the oscillations in the SAN cells: the slow depolarization phase via inward currents ($I_{\text{f}}, I_{\text{CaT}}, [I_{\text{Na}}]$), activation of the inward calcium currents ($I_{\text{CaT}}, I_{\text{CaT}},$), extrusion of calcium ($I_{\text{NaCa}}, I_{\text{CaT}},$). The importance of SR release could lie in the stabilization of the oscillations across frequencies as indicated by Maltsev and Lakatta, but in their article, they also show that the SR release is not actually driving the oscillations (see Figure 5C in their article).

## Insights From Tissue Studies and From Development of the Heart

The studies on which we have commented so far focus at the level of ion channels and the integration of their activity at the level of single cells. The other contributions to this issue make valuable contributions to the debate at the tissue level, particularly concerning the use of imaging with voltage- or calcium-sensitive indicators and the insights that come from studying the development of pacemaker tissues.

These studies show that integrative physiology of pacemaker activity does not end with analysis of cellular activity. In fact, it has been evident since the first electrophysiological mapping work that the sinus node acts as more than just a few thousand cells beating in synchrony. Depending on the

### Table. Sensitivity Analysis of the SAN Period

<table>
<thead>
<tr>
<th></th>
<th>$I_{\text{CaT}}$</th>
<th>$I_{\text{Cat}}$</th>
<th>$I_{\text{NaCa}}$</th>
<th>$I_{\text{BNa}}$</th>
<th>$I_{\text{f}}$</th>
<th>$I_{\text{up}}$</th>
<th>$I_{\text{st}}$</th>
</tr>
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<tbody>
<tr>
<td>Demir</td>
<td>a</td>
<td>−4.00</td>
<td>p</td>
<td>+1.87</td>
<td>p</td>
<td>−2.16</td>
<td>b</td>
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<td>p</td>
<td>+0.70</td>
<td>a</td>
<td>−0.82</td>
<td>p</td>
</tr>
<tr>
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<td>−0.62</td>
<td>p</td>
<td>+1.74</td>
<td>a</td>
<td>+0.04</td>
<td>c</td>
</tr>
<tr>
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<td>a</td>
<td>+1.73</td>
<td>p</td>
<td>+1.13</td>
<td>a</td>
<td>+0.68</td>
<td>a</td>
</tr>
<tr>
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<td>a</td>
<td>+0.10</td>
<td>b</td>
<td>+2.62</td>
<td>p</td>
</tr>
<tr>
<td>Zhang</td>
<td>a</td>
<td>−3.82</td>
<td>p</td>
<td>+1.71</td>
<td>p</td>
<td>+0.87</td>
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Percentage change of period at steady state attributable to a 10% block of currents and pumps in the mathematical models. Full block: a indicates abolishes oscillations; p, changes period only; b, bursting behavior; c, combined block $I_{\text{BNa}}$ and $I_{\text{st}}$ abolishes oscillations.
precise physiological conditions, the apparent origin of pacemaker activity can shift from one region of the node to another.\textsuperscript{15–17} We refer to “apparent origin” because it would be an oversimplification to suppose that the area leading the depolarization uniquely determines what happens (see also elsewhere\textsuperscript{18}). Electric current flows between any two connected cells that are at different potentials, and this current must influence the leading cells (by slowing them down) as much as they influence the follower cells (by speeding them up). The earliest computations of cell-to-cell interaction in the sinus node using parallel computers showed that even very low connectivity (just a few connexin channels between each cell) could synchronize cells with inherently different rhythms.\textsuperscript{19–21} These computations also revealed that the cells with an intrinsically rapid rhythm, located at the periphery of the node, and which would therefore ‘lead’ the depolarization in an isolated node, become the follower cells when the node is connected to the atrium. Boyett and colleagues showed experimentally and computationally\textsuperscript{22} that isolating the sinus node alters the direction of propagation, with the leading cells shifting from the center to the periphery, and more recent work from Boyett and colleagues has further emphasized how the architecture of the node influences its function.\textsuperscript{23} Anatomically and physiologically necessarily interact at the tissue level. A valuable set of insights from the debate between Efimov and Federov versus Joung and Lin in Circulation Research\textsuperscript{13} is that the origin of the impulse is multicentric and that single cells and the intact node react differently to drugs and to genetic changes.

Does work at the tissue level shed any light on the relative roles of membrane and calcium-generated oscillations? This is the central focus of the debate between Efimov and Federov versus Joung and Lin. In principle, using voltage-sensitive markers to search for multiple waveforms could make an important contribution. And indeed, the voltage-sensitive dyes do give results that differ from microelectrode recordings. Efimov and Federov attribute this to the fact that the dyes record from an extended region that can include cells and tissues of different types, and so necessarily give composite results. Against this, Joung and Lin point to the fact that confocal calcium imaging can sometimes show calcium changes leading voltage changes toward the end of the pacemaker depolarization, particularly in the presence of isoproterenol. Dissecting out such a complicated set of interrelationships will be difficult. We concur with the remark that future work will “require close collaboration between the mathematical modelers and experimenters to dissect the role of the individual components”\textsuperscript{13} but would add that “dissect” already biases the analysis. In nonlinear interactive systems, attributing relative roles to different components may be misleading. It is the “integrative” function that matters. As also noted in that study, the different mechanisms work synergistically. Because of nonlinearity, this necessarily means that attribution of the quantitative contributions of individual components depends on the physiological and pathological context. This context includes the fact that information flow between the genome and the phenotype is not one way. The phenotype is not a static product of its genes (reviewed elsewhere\textsuperscript{24,25}). There is feedback downward from the phenotype to control gene expression. Good examples are available in this review series, including, notably, the downregulation of 2 important pacemaker currents, \( I_f \) and \( I_{K_s} \), during atrial tachyarrhythmias. Activity in the atrium can therefore remodel the gene expression profile in the sinus node.

Remodeling of gene expression naturally takes us on to consider the other major contribution to this focused issue of the journal because, as Christoffels et al\textsuperscript{14} show, the development of the heart depends on suppression of gene expression as the embryo develops into the adult. All cardiac myocytes in the early embryo show pacemaker rhythm. The change to the adult forms occurs through repression of the gene expression patterns that develop to enable adult working myocardial cells to differentiate. As a consequence, the adult pacemaker cells resemble those of the early embryo. The identification of the transcriptional repressors involved is therefore an important goal. As Christoffels et al show, this is a rapidly developing area, and it contains the promise that we will eventually know the molecular basis of embryonic development of the heart. These insights will also be important in understanding adult function because there is continuous turnover of gene expression. Variations in expression levels during the turnover of ion channels have recently been shown by Ponard et al\textsuperscript{18} to play a role in heart rate variability using a combination of myocyte cultures and computer modeling.

Conclusions

Experimental data and the in silico modeling work show the complexity of the multifactorial system of SAN excitation. The contribution of \( I_f \) and its importance as a pharmaceutical target has been proven by the successful development of ivabradine and is delineated further in the review article by DiFrancesco\textsuperscript{26} in this review series. Further currents seem to be involved in the depolarization phase (\( I_{Ca} \), mechanosensitive currents\textsuperscript{27} and others) whose relative contributions are still to be determined.

It appears almost obvious that also the fast upstroke at the end of the depolarization phase has a large impact on the depolarization frequency by adjustment of the activation threshold. The evidence presented by Lakatta et al\textsuperscript{15} in this review series underlines also the influence of SR Ca\textsuperscript{2+} release in generation of the upstroke (besides \( I_{CaL} \) and \( I_{CaT} \)), in the extreme showing similarities to delayed afterdepolarizations.

Already, earliest modeling and experimental work showed (consistent with current findings) that neither \( I_f \) nor spontaneous Ca\textsuperscript{2+} release from the SR on its own is or can be driving the pacemaking activity; there is always concerted action necessary and interplay between the various ion channels. Also, the heart rate regulation via cAMP is influenced by and influences multiple ion channels, pumps, and exchangers, thereby creating a robust and stable, but still flexible, system that maintains the billions of heart beats in a normal life.

Future work will most probably discover even further mechanisms influencing heart rate that might be even more relevant targets in specific diseases and pathologies than the currently known pathways.

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