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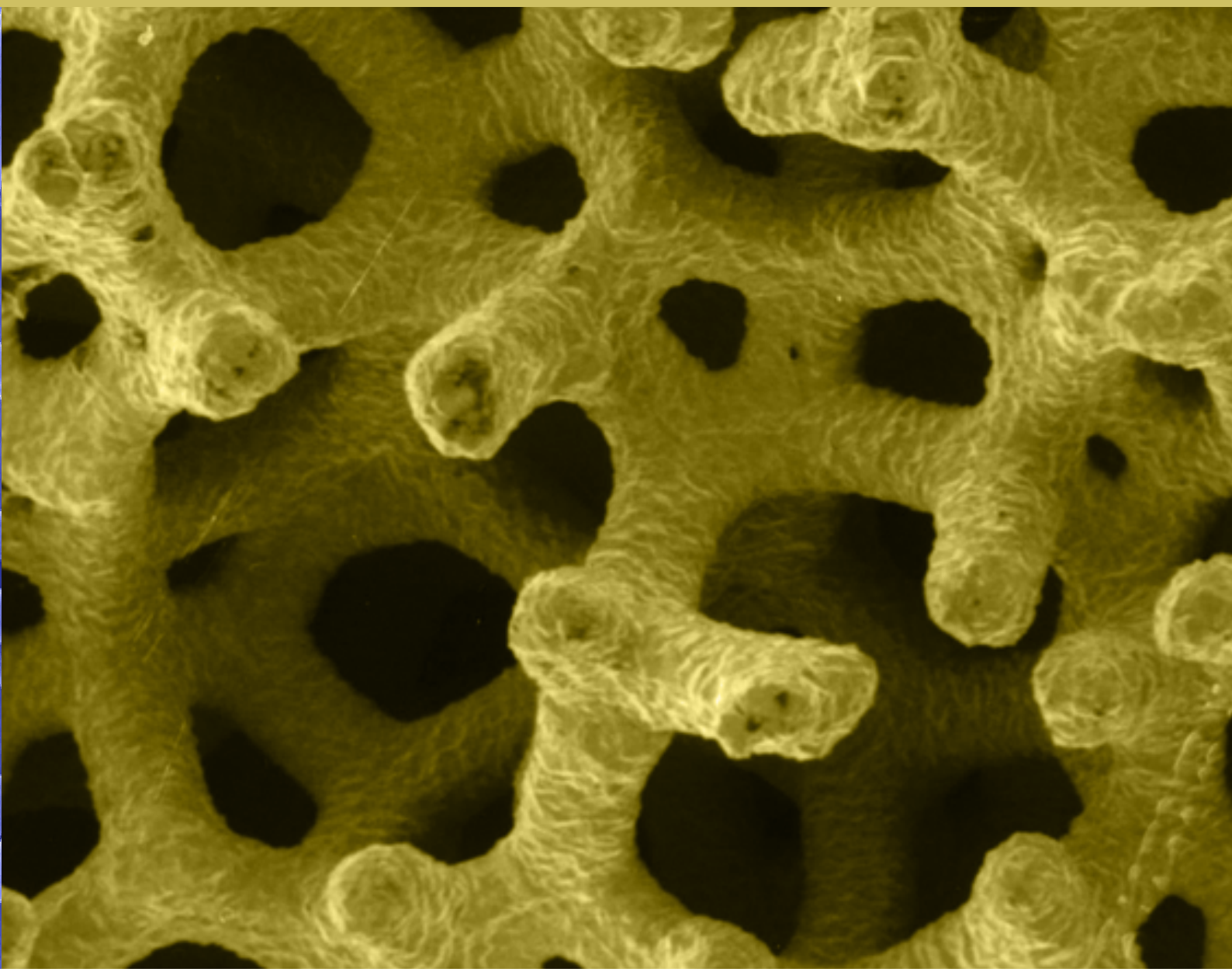

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THE TANGO BETWEEN PKA AND CaMKII SIGNALING IN CARDIAC PACEMAKER CELLS

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ABSTRACT

The sinoatrial node is the primary pacemaker that controls the heart rate under normal conditions. Although the heart rate was originally measured thousands of years ago, the mechanisms that control the spontaneous beating of the sinoatrial node (SAN) are still under debate. In the last century, SAN function was mostly investigated by electrophysiological tools. Therefore, not surprisingly, the major mechanisms that control SAN function were thought to be related only to membranal ionic modulations. Recent biophysical, biochemical and imaging techniques have shed new light on the role of intrinsic pacemaker mechanisms on SAN function. Specifically, the role of post-translational modification signaling on SAN function has been explored using numerical and experimental tools. We describe here the major breakthroughs related to these signaling mechanisms in SAN cells. We conclude that the recent findings are only the tip of the iceberg in the fascinating world of downstream post-translational modification signaling, and we point out future research directions that may increase our knowledge of pacemaker function.

Keywords: Coupled-clock system, Mathematical modeling, Pacemaker.

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Introduction

During hibernation a bear's heart rate can decrease to 10 beats/min [1] while 700 beats/min was recorded in short-tailed shrews [2]. The primary pacemaker in these mammals and others is the sinoatrial node (SAN). Although the heart pulse was originally measured more than thousands of years ago, the mechanisms that control the spontaneous beating of SAN cells are still under debate. The SAN consists of hundreds to thousands (depending on the mammal species) of pacemaker cells that can beat spontaneously even without any neural stimulation. Using recent biophysical, biochemical and imaging techniques, it has been revealed that pacemaker cell function is controlled by internal and membrane mechanisms. Moreover, the identity of the nodes that connect these mechanisms has been revealed. Here, we focus on the role of post-translational modification signaling on SAN function, as discovered using both numerical and experimental approaches. We describe here the major breakthroughs related to these signaling mechanisms in SAN cells.

A coupled-clock system controls pacemaker function

Pacemaker function is orchestrated by two clocks (Fig. 1): the surface membrane clock (M clock), an ensemble of sarcolemmal electrogenic molecules, and the Ca^{2+} clock (the sarcoplasmic reticulum (SR)) (for a review see [3]). A numerical model (for a review see [4]) predicted that these clocks are coupled, as was confirmed by experimental measurements (for a review see [5]). The two clocks are coupled through Ca^{2+} signaling: local Ca^{2+} releases (LCRs) activate an inward Na^{+}/Ca^{2+} exchange (NCX) current and other Ca^{2+} dependent mechanisms (e.g., Ca^{2+}-activated potassium channels [3] and Ca^{2+}-dependent inactivation of L-type Ca^{2+} channels) that prompt the M clock to generate an action potential (AP). By definition, the LCR signal is regulated by the SR Ca^{2+} content and thus by the Ca^{2+} clock. However, because the L-type Ca^{2+} channels regulate cell Ca^{2+} available for SR pumping, the LCR signal is controlled by both clocks, and its magnitude indicates the degree of cross-talk between the clocks [6, 7]. Thus, the Ca^{2+} is a node that connects the two clocks, where an amplification of LCR signal leads to an increase in the spontaneous AP firing rate and vice versa. Ca^{2+} not only directly couples the clocks, but also does so indirectly through the phosphorylation cascade: Ca^{2+} activates calmodulin-adenylyl cyclase (AC)-dependent protein kinase A (PKA) and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) [8, 9]. These phosphorylation signaling cascades act on the same Ca^{2+} (ryanodine receptors and phospholamban) and M-clock proteins (L type Ca^{2+} channels and K^{+} channels). The increase in phosphorylation activity leads to amplification of the LCR Ca^{2+} signal and therefore to an increase in the AP firing rate. Thus, both Ca^{2+} cycling and phosphorylation activity levels indicate the degree of clock coupling: when the signal is further amplified, so are the activity levels of the membrane and SR Ca^{2+} molecules, and thus the AP firing rate is higher as well

to the mammal each group worked with (mice vs. rabbits). Alternatively, it might be related to other non-specific gene changes in these mutant mice (e.g., L-type) that contribute to AP firing.

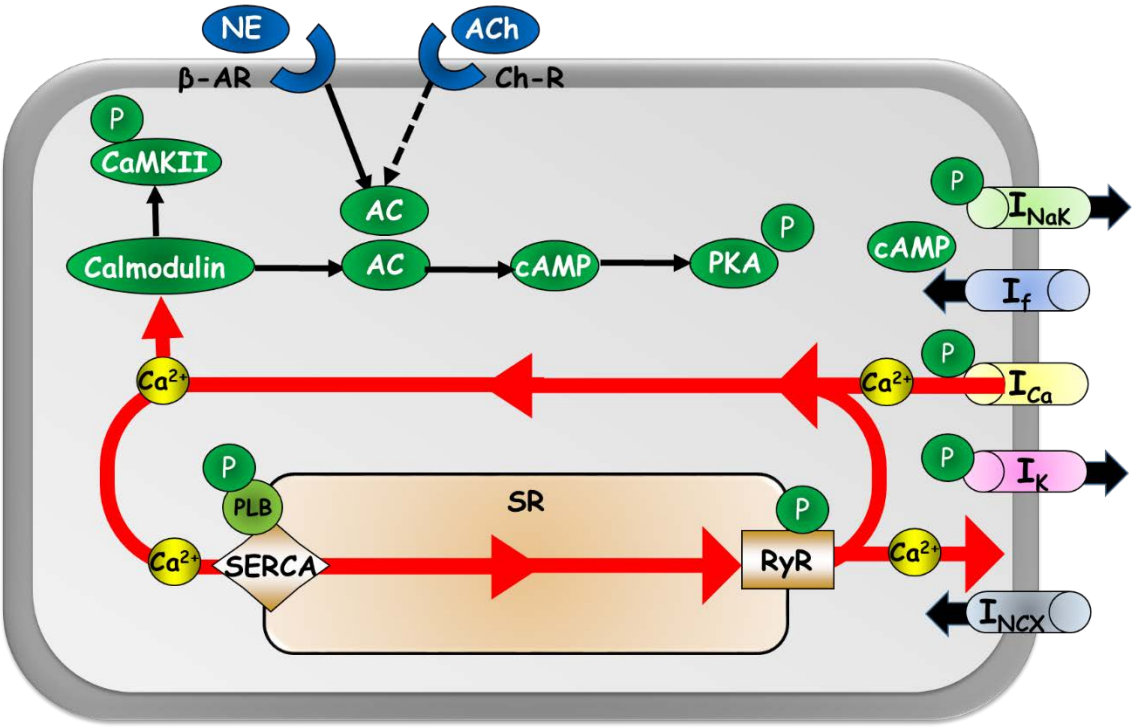

Figure 1. Schematic figure illustrating the cross-talk between M clock (membrane molecules) and the Ca^{2+} clock (sarcoplasmic reticulum (SR)). Two major nodes connect between the clocks: Ca^{2+} and CaMKII-cAMP/PKA signaling. Local Ca^{2+} release through the ryanodine receptor (RyR) increases inward sodium-Ca^{2+} exchange current that ignites together with I_f the action potential. Increase in membrane potential lead to opening of L-type channels that reload the SR. Thus, both clocks components contribute to Ca^{2+} signaling. Ca^{2+} also works as indirect node: it activates calmodulin-adenylyl cyclase (AC)-dependent protein kinase A (PKA) and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII). These phosphorylation signaling cascades act on the same Ca^{2+} (RyR and phospholamban (PLB)) and M-clock proteins (L type Ca^{2+} channels, K^+ channels and NaK pump). Note that a different type of AC is activated by brain receptors.

PKA and CaMKII are coupled

As discussed above, PKA and CaMKII are linked upstream (through Ca^{2+}-calmodulin activation). Because both PKA and CaMKII phosphorylate the same Ca^{2+} and M-clock proteins, and these protein activities affect the Ca^{2+} cycling, PKA and CaMKII are also linked downstream. Thus, theoretically, any change in either PKA or CaMKII activity should affect the other mechanism in steady state. Indeed, when a battery of drugs that disturb the M (I_f blocker) or Ca^{2+} clock (SERCA inhibitor or ryanodine blocker) activity is administered, the AP firing rate is reduced, as is the cAMP level in steady state; the same effect was obtained by administering drugs that disturb the interconnected signaling (PKA inhibitor and Ca^{2+} chelate) or the brain signaling (cholinergic receptor activator) [14] (Fig. 2A). Interestingly, application of CaMKII inhibitors (KN-93 or AIP) leads not only to a reduction in the AP firing rate but also to a reduction in cAMP level (Fig. 2B). Importantly, the relationship between spontaneous AP firing rate and cAMP level in response to the afferent battery of drugs is similar to the relationship between spontaneous AP firing rate and cAMP level in response to the CaMKII blocker [11]

(Fig. 2C). Thus, reduction in CaMKII signaling reduces the Ca^{2+} cycling, leading to a reduction in Ca^{2+}-activated AC activity and thus cAMP-PKA signaling. Theoretically, the opposite cascade from reduction in PKA signaling to reduction in CaMKII activity should exist; however, no experimental tools exist today to prove or disprove this theory.

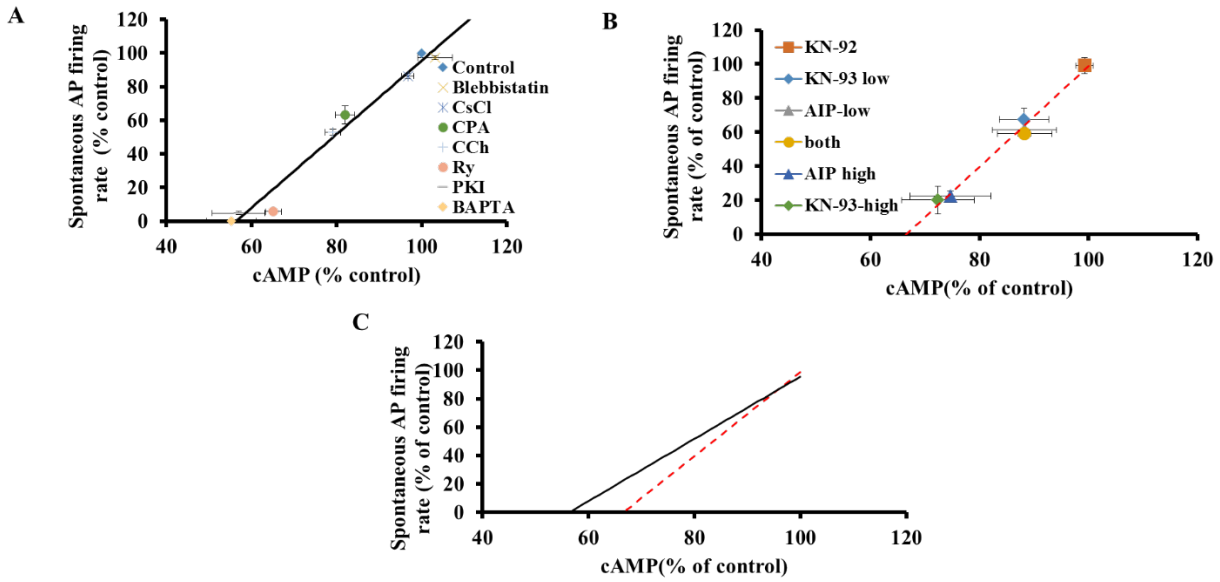

Figure 2. The relationship between spontaneous action potential firing rate and steady state cAMP in response to (A) a battery of drugs: blebbistatin (blocks spontaneous contractions, 10 μM), CsCl (I_f blocker, 2mM), cyclopiazonic acid (inhibiting the sarcoplasmic reticulum Ca^{2+} pump, 5 μM), carbachol (activating cholinergic receptors, 0.2μM), ryanodine (disabling ryanodine receptors, 30μM), PKI (PKA inhibitor, 25μM) and BABTA (buffering intracellular Ca^{2+}, 25 μM); (B) KN-92 (inactivated CaMKII inhibitor, 3 μM), AIP (inhibiting CaMKII, 2 and 10μM), KN-93 (inhibiting CaMKII, 0.5 and 3 μM), and a combination of low AIP and low KN-93 (denoted in the figure as **both**). (C) Note the resemblance between the action potential beating interval in response to the battery of drugs in (A) (black line) and in response to CaMKII inhibition in (B) (red line). Data are from [11, 14].

AC-cAMP-PKA signaling regulates SANC automaticity in all physiological ranges

Although the role of PKA as a key nodal signaling pathway that interacts with both M and Ca^{2+} clock functions has been known for more than 10 years [15], its magnitude, dynamics and spatiotemporal regulation have only recently been described in heart pacemaker cells [16]. In cultured adult rabbit pacemaker cells infected with an adenovirus expressing the FRET sensor AKAR3, the magnitude and time response of PKA activity and the spontaneous AP firing rate were similar in response to graded increases in the intensity of β-adrenergic receptor (β-AR) stimulation (by isoproterenol) or in response to phosphodiesterase (PDE) inhibition. Thus, the kinetics and stoichiometry of increases in PKA activity in response to a physiological (β-AR stimulation) or pharmacological (PDE inhibitor) stimuli match those of changes in the AP firing rate. Recent work has also shown that the kinetics and stoichiometry of decreases in PKA activity in response to a physiological stimulus (cholinergic receptor stimulation) match those of changes in the AP firing rate [17]. Therefore, AC-cAMP-PKA signaling

can play a similar role to that of PKA in pacemaker function. Future experiments on CaMKII activity, using approaches similar to those described here for measuring PKA signaling are needed.

PLB phosphorylation is an important mediator of changes in the spontaneous AP firing rate

Although the experimental results described in the previous section revealed for the first time PKA kinetics and its importance to pacemaker function, the main molecular targets that mediate between PKA signaling and pacemaker function are not known. To identify these targets, a novel mechanistic model was developed [16, 17]. In addition to a description of membrane molecules and internal pacemaker mechanisms, the model includes a description of autonomic-nervous receptors and post-translation signaling cascades. The model reproduces the experiments described in section 4 and yields two important predictions: [1] AC-cAMP-PKA signaling is the core cascade between autonomic receptor stimulation and pacemaker function and [2] directly activating internal clock mechanisms can mimic the effect of autonomic receptor stimulation. However, an even more important prediction was that phospholamban is an important PKA target in allowing the fight-or-flight response, and an important component in maintaining the changes in AP firing rate in response to cholinergic stimulation. A similar conclusion on the important role of phospholamban in mediating the PKA effect on pacemaker function was reached before [18]. However, the model in [18] includes a phenomenological description of AC-cAMP-PKA signaling. Others also attempted to find the main molecular targets that mediate between PKA signaling and pacemaker function [19]. Although the Noma group [19] included in their model a description of cAMP/PKA signaling, they did not include the connection between Ca^{2+} and AC, a description of cholinergic receptor stimulation, and correct Ca^{2+} cycling dynamics.

The authors of [20] numerically modeled the effect of CaMKII level on pacemaker function. The model predicts how SANC death and fibrosis are expressed in myocardial infraction by oxidizing CaMKII affect pacemaker function. However, lack of experimental results on CaMKII dynamics prevents the development of a numerical mechanistic model similar to that described above (reviewed in [21].

Mutations in the coupled clock pacemaker system involve changes in CaMKII-cAMP/PKA signaling

As described above, alteration in CaMKII and/or cAMP/PKA signaling can lead to disturbances in clock function. However, any alteration in one clock mechanism will be transmitted to the other clock mechanism via changes in CaMKII and cAMP/PKA signaling, and all of these together will affect pacemaker function [22]. For example, ivabradine, a specific I_f blocker, can indirectly alter the release of LCR, a coupled-clock function parameter. The numerical model predicts that the ivabradine effect on coupled-clock system function is through the effect on Ca^{2+} cycling and Ca^{2+} activated AC-cAMP/PKA signaling [7]. Similarly, a mutation that increases the funny channel's sensitivity to cAMP (R524Q) leads to a shorter AP firing rate than that of wild-type cells, and this gain in pacemaker function is the net effect of the R514Q mutation on the functioning of the coupled-clock system through Ca^{2+} cycling and Ca^{2+}-activated AC-cAMP/PKA signaling (23). On the other hand, a mutation that induces decrease in I_f function (mutant S672R channels respond normally to cAMP but they are activated at more negative voltages) leads to a longer AP firing rate than that of wild-type cells, and this loss in pacemaker

function is the net effect of the mutation on the functioning of the coupled-clock system through Ca^{2+} cycling and Ca^{2+} activated AC-cAMP/PKA signaling [23]. Thus, AC-cAMP-PKA signaling is involved in both gain and loss of pacemaker cell function.

Summary

PKA and CaMKII signaling are dancing the tango. By means of this dance, they control coupled-clock system function, either by directly changing their dynamics or by transmitting changes from one coupled clock mechanism to the others. Future works will reveal more about the spatial dynamics of PKA and CaMKII signaling in healthy pacemaker cells, in deteriorated pacemaker cells associated with different cardiac diseases and aging [24].

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