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Pacemaker gene mutations, bradycardia, arrhythmias and the coupled clock theory

Yael Yaniv and Edward G. Lakatta

Laboratory of Cardiovascular Science, Biomedical Research Center, Intramural Research Program, National Institute on Aging, NIH, Baltimore, Maryland, USA

To the Editor:

In a recent paper, Nawathe et al.¹ showed that M54T LQTS6 mutation in neonatal rat ventricular myocytes that were co-transfected with human HCN4, the dominate component of f-channels, is associated with bradycardia. The bradycardiac effects that were associated with these mutations, as claimed by the authors, are attributable solely to direct I_f inhibition.

It is well established that normal automaticity of sinoatrial node cell (SANC) is regulated by integrated functions within a system of coupled-clocks. The sarcoplasmic reticulum (SR), traditionally defined as “ Ca^{2+} clock”, spontaneously generates diastolic Ca^{2+} releases that activate an inward current (specifically Na^+ - Ca^{2+} exchanger current (I_{NCX})), that accelerates the rate of diastolic membrane depolarization. The f-channel current (I_f) another member of the “Membrane clock” (i.e., the ensemble of sarcolemmal electrogenic molecules), concurrently drives the diastolic membrane depolarization with I_{NCX} .² The two clocks are tightly coupled by a common chemical axes: Ca^{2+} -calmodulin activated adenylyl cyclases that generates the second messenger cAMP, which activates protein kinase-A (PKA), and Ca^{2+} activated calmodulin-dependent kinase II (CaMKII). Both kinases phosphorylate different proteins of the M clock (e.g., L-type and K^+ currents) and of the Ca^{2+} clock (phospholamban and ryanodine receptors). Additionally, cAMP positively shifts the f-channel activation curve. According to the coupled-clock theory, the membrane and Ca^{2+} clocks should crosstalk. Thus, a change in action potential (AP) firing rate in response to **any** disturbance signal that directly perturbs **either** clock entrains the function of the **other** clock. Consequently, the resultant steady-state AP cycle length change embodies contributions of both clocks. Therefore, the interpretation that bradycardia associated with mutation in HCN4 or with pharmacological blockade of I_f , occurs **solely** on the basis of direct inhibition of I_f function may not be correct. In this regard, a recent study demonstrated that ivabradine, at a concentration that specifically inhibits I_f , but does not directly suppress L-type current, SR Ca^{2+} cycling and other surface membrane ion channels, **indirectly** suppresses intracellular Ca^{2+} cycling.³ Similar clock coupling effects should occur in the presence of HCN4 mutations. A plausible, indirect effect on SR Ca^{2+} in response to HCN4 mutations or pharmacological blockade of I_f can be explained on the basis of this net reduction in Ca^{2+} influx associated with the bradycardic effect (fewer APs per unit time). A reduction in I_f initiates a small reduction in number of APs per unit time, which reduces the Ca^{2+} influx per unit time. This reduction in net Ca^{2+} influx results in a reduction in intracellular Ca^{2+} , and Ca^{2+} available for pumping, reducing the SR Ca^{2+} load and prolonging the restitution time required for spontaneous diastolic Ca^{2+} releases to occur, i.e., the period of local Ca^{2+} release becomes prolonged. Consequently, the Ca^{2+} activation of

I_{NCX} to a later time during diastolic depolarization, which further reduces the heart rate, leading to further reduction in Ca^{2+} influx per unit time. Moreover, as a result of reduced intracellular Ca^{2+} , Ca^{2+} activated CaMKII and adenylyl cyclases-cAMP/PKA signaling axes become damped. The resultant reduction in phosphorylation of Ca^{2+} cycling proteins that further reduces the net Ca^{2+} influx, further reduces SR Ca^{2+} loading that further reduces AP rate and, in parallel, the resultant reduction in cAMP further shifts the I_f activation curve. Thus, the steady-state bradycardia measured experimentally in response to HCN4 mutations or pharmacological blockade of I_f is achieved when the induced reduced Ca^{2+} influx is balanced by a reduction in Ca^{2+} efflux via I_{NCX} .

Similar to mutations in the f-channel within the membrane clock, mutations in the intracellular Ca^{2+} clock proteins are associated with bradycardia. A mutation in ryanodine receptor, axon-3, carried by patients with catecholaminergic polymorphic ventricular tachycardia, is associated with arrhythmias.⁴ Isolated SANC from mice that express this mutation have a slower spontaneous AP firing rate.⁵ Patients with a mutation in CACNA1D, which encodes the pore-forming α_1 subunit of Cav_{1.3} (Cav_{1.3} is largely absent from the ventricles, but is highly expressed in atria, atrioventricular node and sinoatrial node⁶) experience pronounced bradycardia in 12–24-h ECG recordings, and their heart rate variability is increased.⁷ Similar to HCN4 mutations, the steady-state bradycardia associated with mutations in Ca^{2+} clock proteins may be mediated only in part by a reduction in available Ca^{2+} that inhibits Ca^{2+} cycling, and in part, by the resultant changes in cross-talk induced change in the membrane clock.

In summary, crosstalk occurs between the Membrane and Ca^{2+} clocks whenever either the membrane or Ca^{2+} clock is directly perturbed the other clock is indirectly perturbed. Therefore, the steady-state bradycardia associated with different HCN4 mutations, ivabradine or Ca^{2+} regulatory protein is likely mediated, only in part by I_f inhibition, and in part, by change in Ca^{2+} clock due to clock cross-talk. Only an understanding of the full profile underlying mechanisms of bradycardia that is associated with HCN4 mutations will promote the discovery of optionally effective treatments for bradycardia.

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