Mechanism of Pacemaking in \( I_{K1} \)-Downregulated Myocytes

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Biological pacemakers were recently created by genetic suppression of inward rectifier potassium current, \( I_{K1} \), in guinea pig ventricular cells. We simulated these cells by adjusting \( I_{K1} \) conductance in the Luo-Rudy model of the guinea pig ventricular myocyte. After \( 81\% \) \( I_{K1} \) suppression, the simulated cell reached steady state with pacemaker period of 594 ms. Pacemaking current is carried by the \( Na^+-Ca^{2+} \) exchanger, \( I_{NaCa} \), which depends on the intracellular calcium concentration [\( Ca^{2+} \)]. This [\( Ca^{2+} \)] dependence suggests responsiveness (increase in rate) to \( \beta \)-adrenergic stimulation (\( \beta \)AS), as observed experimentally. Simulations of \( \beta \)AS demonstrate such responsiveness, which depends on \( I_{NaCa} \) expression. However, a simultaneous \( \beta \)AS-mediated increase in the slow delayed rectifier, \( I_{Ks} \), limits \( \beta \)AS sensitivity.

Recent experiments demonstrate that cardiac biological pacemakers (BPs) can be created by genetic suppression of inward rectifier potassium current (\( I_{K1} \)) in guinea pig ventricular myocytes.\(^1\) A potential advantage of this approach, as a therapeutic alternative to electronic pacemaking, is possible responsiveness to regulatory inputs, eg, \( \beta \)-adrenergic stimulation (\( \beta \)AS).

To advance this technology, it is important to understand the BP pacemaking mechanism. In the present study, we demonstrate that \( Na^+-Ca^{2+} \) exchanger (\( I_{NaCa} \)) is the pacemaker current and explore BP responsiveness to \( \beta \)AS.

Materials and Methods

The Luo-Rudy (LRd) guinea pig ventricular myocyte model\(^2\) was used to investigate BP pacemaking. Two \( I_{K1} \) suppression levels (\( 81\% \) and \( 100\% \)) and \( I_{NaCa} \) expression levels (control and \( 100\% \) increase) were simulated. \( \beta \)AS effects were simulated\(^3\) based on experimental observations. Abbreviations are defined in the Figure legend.

An expanded Materials and Methods section can be found in the online data supplement available at http://www.circresaha.org.

Results

After \( 81\% \) \( I_{K1} \) suppression, we observe spontaneous action potentials (APs) that, after a 16-second transition, settle into a stable oscillatory pattern (Figure, panel A). Activity is initiated by slow depolarization generated by sodium and calcium leakage (background currents) and \( I_{NaCa} \), that extrudes calcium to maintain homeostasis at rest.\(^2\) In unmodified cells (intact \( I_{K1} \)), these inward currents are balanced by outward \( I_{K1} \), and resting \( V_m \) is stable.\(^4\) In the BP cell, when \( V_m \) reaches \(-60 \) mV, \( I_{Na} \) activates and increases depolarization rate. Peak \( I_{Na} \) is two orders of magnitude smaller than that of a paced AP\(^2\) due to inactivation during the slow depolarization (Figure, panel B). \( I_{Na} \) and initial activation of \( I_{CaL} \) continue depolarizing \( V_m \) as \( I_{NaCa} \) decreases (higher \( V_m \) reduces its driving force). T-type calcium current (\( I_{CaT} \)) does not contribute because of inactivation during the slow depolarization (in ventricular myocytes these channels are unavailable at potentials above \(-65 \) mV).\(^5\) Once \( I_{CaL} \) is fully activated, it supports the subsequent upstroke and plateau of the AP (Figure, panel E). \( dV_m/dt \) corresponds to peak \( I_{CaL} \) and is much smaller than that of \( I_{Na} \)-dependent paced APs (15 V/s versus 388 V/s).\(^2\) As \( I_{K1} \) and \( I_{Ks} \) repolarize \( V_m \), \( I_{NaCa} \) driving force increases, causing larger inward current (Figure, panel C). At \(-67.8 \) mV (maximum diastolic potential, MDP, Figure, panel A), outward \( I_{Ks} \), \( I_{Na} \), and the suppressed \( I_{K1} \) (Figure, panel H) do not balance inward \( I_{NaCa} \) and background currents. This imbalance causes slow phase-4 depolarization (\( \phi \)d4) that leads to generation of a subsequent AP and continuous pacemaking.

Pacemaking mechanism remains similar during steady state. While removing residual \( Ca^{2+} \) from calcium-induced calcium release (CICR) of the previous AP, \( I_{NaCa} \) generates inward current that, in absence of balancing \( I_{K1} \), depolarizes \( V_m \) to AP threshold. During sustained oscillations, there is higher [\( Ca^{2+} \)], due to loading (Figure, panel D). Increased [\( Ca^{2+} \)] affects rate by augmenting forward-mode \( I_{NaCa} \) (inward current) during \( \phi \)d4 (Figure, panel C), which accelerates depolarization (Figure, panel A). At the end of \( \phi \)d4, as \( I_{NaCa} \) decreases, \( I_{Ks} \) transiently increases and depolarizes \( V_m \) to threshold for \( I_{CaL} \) activation, which generates the AP upstroke. At the end of the AP (beginning of \( \phi \)d4), \( I_{Ks} \) is still partially activated (Figure, panel G) and is important in determining MDP and rate of early \( \phi \)d4. \( I_{Ks} \) expression also affects the rate of \( \phi \)d4, \( 81\% \) \( I_{K1} \) suppression results in oscillations at cycle length (CL) of 594 ms (Figure, panel A), and complete \( I_{K1} \) suppression leads to much faster rate (CL=366 ms, not shown).

Changes in pacemaker rate under \( \beta \)AS are investigated by modifying AP currents according to their experimental response to \( \beta \)-agonists (see Reference 3 in the online data supplement). Enhanced \( I_{Na} \) (\( Ca^{2+} \) uptake by the sarcoplasmic reticulum, SR), \( I_{CaL} \), or \( I_{NaK} \) (the \( Na^+-K^+ \) pump) accelerates rate. Increased \( I_{Ks} \) or negative shift of \( I_{NaCa} \) inactivation decreases rate. The Table provides data for individual protocols.
Selected processes during spontaneous initiation (first two APs) and steady-state oscillations in BP cells. 

and their combined effect. Increasing \( I_{Na} \) (110%) in the control BP cell (81% \( I_{K1} \) suppression) results in a 24% rate increase. This increase corresponds to SR loading and increased \( [Ca^{2+}] \), that augments forward \( I_{NaCa} \). Similarly, increasing \( I_{Ca,L} \) (300%) increases rate (11%) by augmenting \( [Ca^{2+}] \), and \( I_{NaCa} \). This increase occurs despite \( I_{Ca,L} \)-mediated increase in AP duration (APD), which decreases rate. Contrary to expectation, increasing \( I_{NaCa} \) (an outward current) also increases rate. Augmenting \( I_{NaCa} \) increases the sodium gradient, which increases \( I_{Na} \) and \( I_{NaCa} \) by increasing their driving force, accelerating \( \phi 4d \). Because of \( I_{Na} \) participation during \( \phi 4d \), negative shift of its inactivation decreases rate. This effect abolishes \( I_{Na} \) in BP cells, in contrast to cells with intact \( I_{K1} \) where \( I_{Na} \) is only reduced by 14%. \( I_{K1} \) increase by \( \beta AS \) decreases APD, which accelerates rate. However, it also hyperpolarizes \( V_m \) to a more negative MDP, which prolongs \( \phi 4d \) to the next AP threshold. The net effect is slowing of rate (13%), indicating that effects on MDP dominate APD changes. The overall effect of \( \beta AS \) on rate (only 4% increase) is very small, indicating low BP sensitivity to \( \beta AS \). However, the simulated control BP cell is epicardial, which expresses relatively low \( I_{NaCa} \) density (average midmyocardial is 50% higher). When \( I_{NaCa} \) density is increased 100% (estimated upper limit), \( \beta AS \) causes a 24% rate increase (Table). Note that all other model parameters were kept constant, to study the isolated effect of \( I_{NaCa} \) expression. We conclude that the \( \beta AS \) sensitivity of BP cells depends strongly on \( I_{NaCa} \) expression levels.

BP cells show increased \( [Ca^{2+}] \), at steady state compared with paced cells at the same CL (1.22 and 0.94 \( \mu \)mol/L, respectively, Figure, panel D). \( [Ca^{2+}] \), is further increased by \( \beta AS \) and by rate increases, which could cause calcium overload. We test this possibility by applying \( \beta AS \) to a rapidly paced cell (\( I_{K1} \) fully suppressed; control \( I_{NaCa} \)) and comparing \( [Ca^{2+}] \), to that of a slower BP cell (81% \( I_{K1} \) suppression; control \( I_{NaCa} \)) without \( \beta AS \), finding 85% increase in peak \( [Ca^{2+}] \), (from 1.22 to 2.25 \( \mu \)mol/L). This result suggests that, from this perspective, increasing \( I_{NaCa} \) expression would be a preferred method of increasing \( \beta AS \) sensitivity, because of enhanced calcium removal capacity and protection against calcium overload.

The modulatory role of calcium in pacemaking suggests that \( I_{Ca,L} \) antagonists may overly suppress pacemaking in BP cells. We simulate this effect by 50% \( I_{Ca,L} \) block and observe 18.7% decrease in CL, in the range observed for similar block in sinoatrial node (SAN) cells.

**Discussion**

In a recent study, viral gene transfer was used to convert quiescent myocardial cells into pacemaker cells. With \( \approx 80\% \) of \( I_{K1} \) channels suppressed, these cells generated a rhythmic excitation at an intrinsic CL of 600 ms. The spontaneous APs were initiated by slow \( \phi 4d \) from MDP of \(-60.7 \pm 2.1 \) mV.

Similar behavior is observed in the computer simulations; when \( I_{K1} \) is suppressed by 81%, stable oscillatory behavior is
attained. Slow φ4d from MDP of −67.3 mV sustains rhythmic excitation at a CL of 594 ms. Complete Ik, suppression increases the rate to a CL of 366 ms, implying that altering Ik, expression levels could be used to set intrinsic BP cell pacemaker rate.

The simulations identify IsCa as the regulated membrane process responsible for φ4d and pacemaking. Large Ik conductance determines resting Vm, which is close to K+ reversal potential. When Ik+ is suppressed, the steady-state balance between inward and outward currents shifts in the inward direction. The most important currents at this phase are involved with [Ca2+]i homeostasis: calcium leakage that brings calcium into the cell and IsCa, that extrudes calcium. These inward currents depolarize Vm to initiate a spontaneous AP. After this AP, residual [Ca2+]i, from CICR determines the magnitude of IsCa, and consequently the rate of diastolic depolarization. At steady state, CICR (triggered by Ica,L) generates similar [Ca2+]i transients every beat, resulting in a similar φ4d rate between APs and stable pacemaking at constant rate. This mechanism differs from spontaneous activity where spontaneous SR calcium release, an irregular process, underlies AP generation.8 This distinction is essential to the regular rhythm generated by BP cells, a prerequisite for any functional pacemaker.

An important determinant of IsCa is [Ca2+]i, which enhances its forward mode. This property links βAS to pacemaker current and may explain why it accelerates with isoprenaline.1 However, because of simultaneous βAS-mediated Ik, increase and IsCa, reduction, our simulations suggest that BP responsiveness to βAS is very limited (quantitative data are not provided in Reference 1) and strongly depends on IsCa, expression. For simulated high IsCa, density, βAS increases the rate by 24%. For comparison, 115% increase is observed in isolated SAN cells,9 indicating much greater responsiveness to βAS.

The role of [Ca2+]i, in modulating CL suggests further experimental investigation. βAS can cause excessive Ca2+ SR loading and spontaneous release during φ4d, interrupting the regular rhythm. At fast rates with strong βAS, simulated peak [Ca2+]i increases 85% compared with only 50% for SAN cells.9 Therefore, experiments examining a range of βAS are required to determine [Ca2+]i, overload levels and likelihood of arrhythmic APs. Ca2+ overload is also likely to induce long-term electrophysiological remodeling, which should be considered. Additionally, the model prediction that Ica,L antagonists will have similar effects in BP and SAN cells should be confirmed.

There are other mechanistic differences between BP and SAN cells. BP cells rely on a single dominant membrane process, IsCa, as the carrier of pacemaker current causing φ4d. Nodal cells rely on several depolarizing currents for φ4d and pacemaking. These include Ina,T, Ii (the hyperpolarization-activated current), IsCa, Ica,L, and possibly Ina (a sustained inward current, see review10). This multiplicity provides many control points for pacemaking regulation by various (neural and other) inputs. As suggested,10 this multiplicity underlies spatial heterogeneity within the SAN structure, which may be important for its function. In addition, Ik,ac+ an acetylcholine-sensitive current not detected in ventricular myocytes, provides vagal control of SAN rate. Finally, SAN ability to drive the heart depends on its architecture (gap-junction distribution; branching fibers), which facilitates optimization of its electrical loading by the surrounding atrial tissue. Therefore, it should be recognized that the engineering of single BP cells is only a first step toward creation of functional BP complexes.

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References


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