Published in final edited form as:
*Circ Res*. 2003 February 21; 92(3): 261–263.

# Mechanism of Pacemaking in $I_{K1}$-Downregulated Myocytes

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## Abstract

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 $[Ca^{2+}]_i$. This $[Ca^{2+}]_i$ 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.

### Keywords

pacemaker; arrhythmias; ion channels; gene therapy

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[2] 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.

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This manuscript was sent to Michael R. Rosen, Consulting Editor, for review by expert referees, editorial decision, and final disposition.

## 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 $I_{Ca,L}$ continue depolarizing $V_m$ as $I_{NaCa}$ decreases (higher $V_m$ reduces its driving force). T-type calcium current ($I_{Ca,T}$) does not contribute because of inactivation during the slow depolarization (in ventricular myocytes these channels are unavailable at potentials above −65 mV).[5] Once $I_{Ca,L}$ is fully activated, it supports the subsequent upstroke and plateau of the AP (Figure, panel E). $dV_m/dt_{max}$ corresponds to peak $I_{Ca,L}$ and is much smaller than that of $I_{Na}$-dependent paced APs (15 V/s versus 388 V/s).[2] As $I_{Kr}$ 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 $I_{Kr}$, $I_{Ks}$, and the suppressed $I_{K1}$ (Figure, panel H) do not balance inward $I_{NaCa}$ and background currents. This imbalance causes slow phase-4 depolarization ($\varphi$4d) 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 $[Ca^{2+}]_i$ due to loading (Figure, panel D). Increased $[Ca^{2+}]_i$ affects rate by augmenting forward-mode $I_{NaCa}$ (inward current) during $\varphi$4d (Figure, panel C), which accelerates depolarization (Figure, panel A). At the end of $\varphi$4d, as $I_{NaCa}$ decreases, $I_{Na}$ transiently increases and depolarizes $V_m$ to threshold for $I_{Ca,L}$ activation, which generates the AP upstroke. At the end of the AP (beginning of $\varphi$4d), $I_{Ks}$ is still partially activated (Figure, panel G) and is important in determining MDP and rate of early $\varphi$4d. $I_{K1}$ expression also affects the rate of $\varphi$4d; 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 $I_{up}$ ($Ca^{2+}$ uptake by the sarcoplasmic reticulum, SR), $I_{Ca,L}$, or $I_{NaK}$ (the $Na^+$-$K^+$ pump) accelerates rate. Increased $I_{Ks}$ or negative shift of $I_{Na}$ inactivation decreases rate. The Table provides data for individual protocols and their combined effect. Increasing $I_{up}$ (110%) in the control BP cell (81% $I_{K1}$ suppression) results in a 24% rate increase. This increase corresponds to SR loading and increased $[Ca^{2+}]_i$ that augments forward $I_{NaCa}$. Similarly, increasing $I_{Ca,L}$ (300%) increases rate (11%) by augmenting $[Ca^{2+}]_i$ and $I_{NaCa}$. This increase occurs despite $I_{Ca,L}$-mediated increase in AP duration (APD), which decreases rate. Contrary to expectation, increasing $I_{NaK}$ (an outward current) also increases rate. Augmenting $I_{NaK}$ increases the sodium gradient, which increases $I_{Na}$ and $I_{NaCa}$ by increasing their driving force, accelerating $\varphi$4d. Because of $I_{Na}$ participation during $\varphi$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%. $I_{Ks}$ increase by $\beta$AS decreases APD, which accelerates rate. However, it also hyperpolarizes $V_m$ to a more negative MDP, which prolongs $\varphi$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,[2] which expresses relatively low $I_{NaCa}$ density (average midmyocardial is 50% higher).[6] 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 $[Ca^{2+}]_i$ at steady state compared with paced cells at the same CL (1.22 and 0.94 $\mu$mol/L, respectively, Figure, panel D). $[Ca^{2+}]_i$ 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 $[Ca^{2+}]_i$ to that of a slower BP cell (81% $I_{K1}$ suppression; control $I_{NaCa}$) without $\beta$AS, finding 85% increase in peak $[Ca^{2+}]_i$ (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.[7]

## Discussion

In a recent study,[1] viral gene transfer was used to convert quiescent myocardial cells into pacemaker cells. With ~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 $\varphi$4d from MDP of −60.7 ± 2.1 mV.

Similar behavior is observed in the computer simulations; when $I_{K1}$ is suppressed by 81%, stable oscillatory behavior is attained. Slow $\varphi$4d from MDP of −67.3 mV sustains rhythmic excitation at a CL of 594 ms. Complete $I_{K1}$ suppression increases the rate to a CL of 366 ms, implying that altering $I_{K1}$ expression levels could be used to set intrinsic BP cell pacemaker rate.

The simulations identify $I_{NaCa}$ as the regulated membrane process responsible for $\varphi$4d and pacemaking. Large $I_{K1}$ conductance determines resting $V_m$, which is close to $K^+$ reversal potential. When $I_{K1}$ 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 $[Ca^{2+}]_i$ homeostasis: calcium leakage that brings calcium into the cell and $I_{NaCa}$ that extrudes calcium. These inward currents depolarize $V_m$ to initiate a spontaneous AP. After this AP, residual $[Ca^{2+}]_i$ from CICR determines the magnitude of $I_{NaCa}$ and consequently the rate of diastolic depolarization. At steady state, CICR (triggered by $I_{Ca,L}$) generates similar $[Ca^{2+}]_i$ transients every beat, resulting in a similar $\varphi$4d 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 $I_{NaCa}$ is $[Ca^{2+}]_i$, which enhances its forward mode. This property links BP rhythm to $\beta$AS and may explain why it accelerates with isoprenaline.[1] However, because of simultaneous $\beta$AS-mediated $I_{Ks}$ increase and $I_{Na}$ reduction, our simulations suggest that BP responsiveness to $\beta$AS is very limited (quantitative data are not provided in Reference 1) and strongly depends on $I_{NaCa}$ expression. For simulated high $I_{NaCa}$ density, $\beta$AS increases the rate by 24%. For comparison, 115% increase is observed in isolated SAN cells,[9] indicating much greater responsiveness to $\beta$AS.

The role of $[Ca^{2+}]_i$ in modulating CL suggests further experimental investigation. $\beta$AS can cause excessive $Ca^{2+}$ SR loading and spontaneous release during $\varphi$4d, interrupting the regular rhythm. At fast rates with strong $\beta$AS, simulated peak $[Ca^{2+}]_i$ increases 85% compared with only 50% for SAN cells.[9] Therefore, experiments examining a range of $\beta$AS are required to determine $[Ca^{2+}]_i$ overload levels and likelihood of arrhythmic APs. $Ca^{2+}$ overload is also likely to induce long-term electrophysiological remodeling, which should be considered. Additionally, the model prediction that $I_{Ca,L}$ 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, $I_{NaCa}$, as the carrier of pacemaker current causing $\varphi$4d. Nodal cells rely on several depolarizing currents for $\varphi$4d and pacemaking. These include $I_{Ca,T}$, $I_f$ (the hyperpolarization-activated current), $I_{NaCa}$, $I_{Ca,L}$, and possibly $I_{st}$ (a sustained inward current, see review[10]). 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, $I_{K,Ach}$, 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.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

#### Acknowledgements

This work was supported by NIH grants R01-HL49054 and R37-HL33343 (to Y.R.) and F31-HL68318 (to J.S.).

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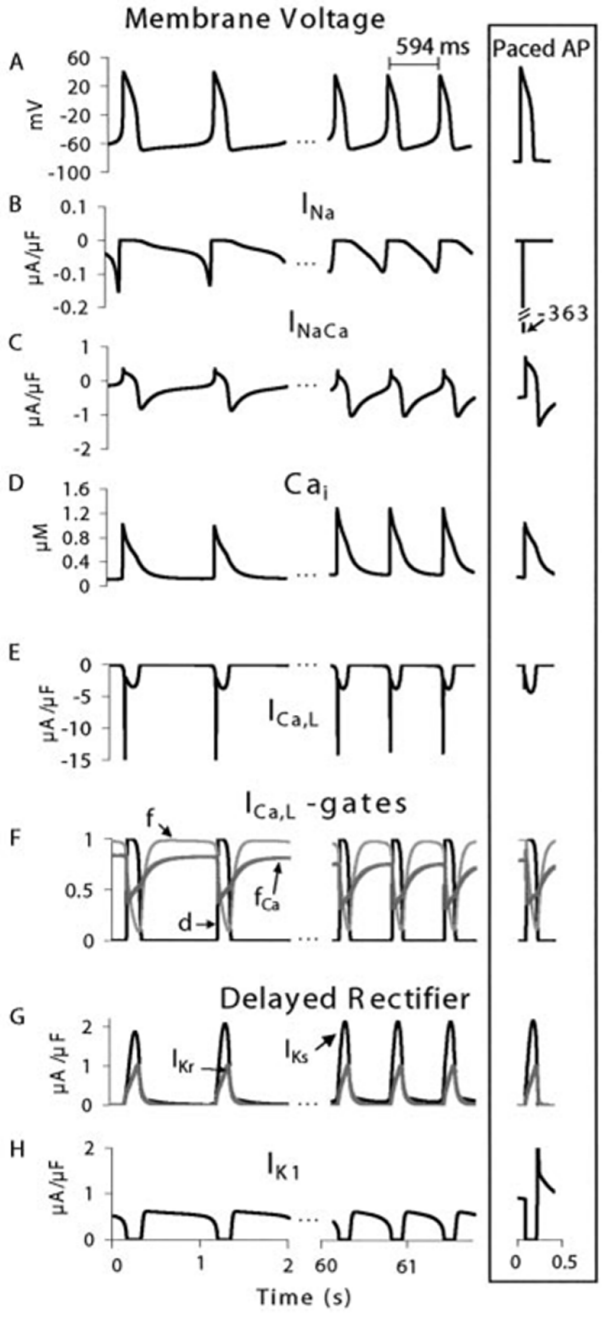


**1.**

Selected processes during spontaneous initiation (first two APs) and steady-state oscillations in BP cells. $I_{K1}$ suppressed by 81%. Paced AP (same CL) is shown for reference (framed right column). A, Membrane potential, $V_m$. B, Sodium current, $I_{Na}$. C, $Na^+$-$Ca^{2+}$ exchange current, $I_{NaCa}$. D, Intracellular calcium, $[Ca^{2+}]_i$. E, L-type calcium current, $I_{Ca,L}$. F, $I_{Ca,L}$ gating: activation gate (d), voltage-dependent inactivation (f), and calcium-dependent inactivation ($f_{Ca}$). G, Fast delayed rectifier $K^+$ current, $I_{Kr}$, and slow delayed rectifier $K^+$ current, $I_{Ks}$. H, Inward rectifier potassium current, $I_{K1}$.

## Relationship Between Pacemaking Rate and Currents Affected by $\beta$AS

| | CL, ms | Frequency, bpm | Percent Change |
|---|---|---|---|
| Control (81% $I_{K1}$ suppression) | 594 | 101 | 0 |
| $I_{up}$ increase (110%) | 480 | 125 | 24 |
| $I_{Ca,L}$ increase (300%) | 548 | 109 | 8 |
| $I_{NaK}$ pump increase (20%) | 549 | 109 | 8 |
| $I_{Ks}$ increase (60%) | 682 | 88 | −13 |
| $I_{Na}$ shift of inactivation (33.4 mV) | 636 | 94 | −7 |
| Total $\beta$AS (all of the above) | 570 | 104 | 4 |
| $I_{NaCa}$ increase (100%) | 567 | 106 | 5 |
| $\beta$AS with $I_{NaCa}$ increase (100%) | 481 | 125 | 24 |

Results of modulating each individual process are shown followed by their cumulative effect (total $\beta$AS). Total $\beta$AS is also simulated with increased $I_{NaCa}$ expression (bottom row), showing dramatic increase in sensitivity. Changes are relative to control.