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# Mechanisms of sinoatrial node pacemaking: novel insights into roles of the pacemaker current $I_f$ from bifurcation analysis of mathematical models

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**Abstract**

To elucidate dynamical mechanisms of sinoatrial node (SAN) pacemaking with special focus on roles of hyperpolarization-activated current ($I_f$), we investigated influences of $I_f$ on parameter-dependent stability and bifurcations of SAN cells, whether blocking $I_f$ abolishes SAN pacemaking, and effects of $I_f$–dependent changes in intracellular $Na^+$ concentration ($Na_i$). Bifurcation analyses were performed for mathematical models of rabbit SAN cells. We conclude that 1) blocking $I_f$ abolishes SAN pacemaking only when cells are hyperpolarized; 2) overlarge $I_f$ does not enhance but attenuates robustness of SAN cells; and 3) enhancing effect of $I_f$ on SAN robustness is reversed by elevations in $Na_i$.

## 1. Introduction

Mechanism of sinoatrial node (SAN) pacemaking is one of the most important subjects to be elucidated in cardiac electrophysiology. Ionic mechanisms of SAN pacemaking have thoroughly been studied experimentally and theoretically. By the theoretical approach using mathematical models for rabbit SAN cells, we provided significant insights into dynamical mechanisms of SAN pacemaking and roles of sarcolemmal ionic currents such as the L-type $Ca^{2+}$ channel current ($I_{CaL}$), delayed-rectifier $K^+$ channel current ($I_K$) and $Na^+$ channel current ($I_{Na}$) [1,2].

Hyperpolarization-activated cation current ($I_f$) contributes to prevention of excess hyperpolarization [3], autonomic regulations of spontaneous activity [4], stabilization of pacemaker frequency [5], and diastolic depolarization in the periphery of intact SAN [6]. However, $I_f$ blockers did not abolish spontaneous activity of real SAN cells [6], suggesting that $I_f$ is not indispensable for spontaneous firings under normal conditions. Thus, the roles of $I_f$ in SAN pacemaking remained to be determined by the theoretical approach.

The aim of our study was to provide more profound insights into the roles of $I_f$ in SAN pacemaking in terms of nonlinear dynamics and bifurcation theory. Initiation and cessation of pacemaker activity are considered as bifurcation phenomena; bifurcation analysis provides an efficient way of understanding how individual currents contribute to pacemaker activities [1,2]. In this study, therefore, we performed bifurcation analyses for mathematical models of the central and peripheral SAN cells [2,8,9]. Bifurcation diagrams were constructed by calculating equilibrium points (EPs), limit cycles (LCs), their stability, and bifurcation points as functions of model parameters. We focused on the effects of $I_f$ on the stability of EPs and robustness of pacemaker activity against hyperpolarizing loads, and thus evaluated stability and dynamics of the model cells during injections of hyperpolarizing bias currents ($I_{bias}$), applications of acetylcholine (ACh) or electrotonic modulations by the atrium. Furthermore, we explored whether and how $I_f$-dependent pacemaking, defined as the pacemaker activity to be abolished by blocking $I_f$, is possible. $I_f$ effects were tested for both the intracellular $Na^+$ concentration ($Na_i$)-fixed system and $Na_i$-variable system. This study provides significant insights into the contributions of $I_f$ to EP instability and robustness of SAN pacemaking as well as how $Na_i$ influences $I_f$ effects.

## 2. Methods

### 2.1. Mathematical Formulation

*2.1.1. Base models for central and peripheral SAN cells*

We used the Kurata et al central [8] and peripheral [2] cell models, and the Maltsev-Lakatta model [9]. These models include 14 membrane current components. The membrane current system includes $I_{CaL}$, $I_{Na}$, $I_f$, T-type $Ca^{2+}$ channel current ($I_{CaT}$), sustained inward current ($I_{st}$), rapidly-activating ($I_{Kr}$) and slowly-activating ($I_{Ks}$) components of $I_K$, 4-AP-sensitive currents consisting of transient and sustained components, background currents carried by $Na^+$ and $K^+$, muscarinic $K^+$ channel current ($I_{KACh}$), $Na^+$-$K^+$ pump current ($I_{NaK}$), and $Na^+/Ca^{2+}$ exchanger current ($I_{NCX}$).

*2.1.2. Incorporation of ACh effects on ionic currents*

To investigate the bifurcation phenomena in the model cells during applications of ACh, we incorporated the formulas of Zhang et al [10] for $I_{KACh}$ and modifications of $I_{CaL}$ and $I_f$ by ACh, into the base models. $I_{KACh}$ density was assumed to be the same in central and peripheral cells.

*2.1.3. Formulation of a coupled-cell model*

We employed a coupled-cell model to investigate the electrotonic influences of atrial myocytes on stability and dynamics of SAN cells. A peripheral SAN cell model was connected to a passive membrane model for an atrial myocyte via the gap junction conductance ($G_C$) of 0–1000 nS. We used the capacitance of 134 pF and resistance of 100–900 MΩ for the atrial membrane model. A resting potential of the atrial myocyte was set equal to −80 mV.

### 2.2. Bifurcation Analysis

The model cells are 15- and 29-order autonomous continuous-time dynamical systems. Dynamical properties of model systems were determined by handling a set of 15 or 29 first-order, nonlinear ordinary differential equations. Numerical computations were performed with MATLAB 7.5 (The MathWorks, Natick, MA, USA).

Bifurcation parameters chosen in this study include 1) the maximum conductance of $I_f$ ($g_f$); 2) amplitude of hyperpolarizing $I_{bias}$; 3) ACh concentration ([ACh]); 4) $G_C$ and 5) $Na_i$. Detailed procedures for locating EPs and LCs, constructing one- and two-parameter bifurcation diagrams (BDs), and detecting bifurcations (determination of EP and LC stabilities) are provided previously [2]. We used 1) Newton-Raphson algorithm to locate EPs and to detect bifurcations of EPs; 2) brute-force approach using a MATLAB ODE solver, *ode15s*, to calculate stable LCs and arrhythmic dynamics; and 3) CL_MATCONT, a *continuation toolbox* for MATLAB, to locate unstable LCs and detect bifurcations of LCs. Types of LC bifurcations were determined by calculating characteristic multipliers.

## 3. Results

### 3.1. Influences of $I_f$ on Bifurcations of SAN Cells

*3.1.1. Effect on bifurcation during ACh application*

We first examined the effects of $I_f$ on bifurcation phenomena during ACh applications in the model cells. One-parameter BDs to illustrate stability and oscillation dynamics of the model cells were constructed as functions of [ACh] for different $g_f$ values. We also constructed two-parameter BDs where $g_f$-dependent changes in Hopf bifurcation (HB) and saddle-node bifurcation (SNB) points were plotted; the critical [ACh] at which oscillation dynamics became arrhythmic via LC bifurcations were determined as functions of $g_f$. During [ACh] increases, EPs of the central cell were stabilized via HBs. Under the normal condition, LCs were destabilized via a period-doubling bifurcation (PDB); spontaneous firings became arrhythmic, abruptly shrunk in amplitude via another PDB, and finally vanished at the HB. In the $I_f$-removed cell, a LC became unstable via a Neimark-Sacker bifurcation (NSB) with emergence of arrhythmic dynamics. EPs of the peripheral cell were also stabilized via HBs during [ACh] increases. In the normal cell, LCs became unstable via NSBs, with arrhythmic dynamics emerging at these bifurcations. The critical [ACh] values to yield arrhythmic dynamics or quiescence became smaller as $g_f$ decreased.

*3.1.2. Effect on bifurcation during electrotonic modulation*

We further examined the effects of $I_f$ on bifurcations during $G_C$ increases of the coupled-cell model. With increasing $G_C$ increases, EPs of the peripheral cell were stabilized via HBs, with the cell coming to a rest. In both the normal and $I_f$-reduced cells, LCs were destabilized via NSBs with the emergence of arrhythmic dynamics.

### 3.2. Combined Effects of $I_f$ and Other Currents

$I_{st}$ and $I_{Na}$, present at high density in the central and peripheral cells, respectively, may play pivotal roles in SAN pacemaking. Therefore, we examined the combined effects of $I_f$ and $I_{st}$ or $I_{Na}$ on bifurcation phenomena during hyperpolarization in the model cells. In the $I_{st}$-removed system, the critical [ACh] value to yield a stable EP was relatively low, with $g_f$ increase not significantly enlarging the [ACh] region of unstable EP. The $I_f$-induced enlargements of the [ACh] and $G_C$ region of unstable EPs were much greater in the periphery than in the center. The removal of $I_{Na}$ shrunk the [ACh] and $G_C$ region of unstable EPs. In the $I_{Na}$-removed system, the $G_C$ region of unstable EPs was not significantly enlarged by $g_f$ increase.

### 3.3. Searching for $I_f$-dependent Pacemaking

In the central cell under the normal condition, reducing $I_f$ did not yield a SNB or HB at which a stable EP emerges, not abolishing LCs. Under the hyperpolarized conditions, however, blocking $I_f$ led to 1) de novo creation of EPs at more negative potentials via SNBs, 2) destabilization of LCs via a PDB with emergence of period-2 periodic and chaotic dynamics, and 3) cessation of spontaneous activity via HBs. In the peripheral cell under the normal condition, reducing $I_f$ did not cause EP stabilization or abolition of LCs. In the cell hyperpolarized by ACh applications, however, blocking $I_f$ caused 1) negative shifts of steady-state potential ($V_E$) with its stabilization via HBs, 2) destabilization of LCs via PDBs, and 3) cessation of spontaneous activity at the HB points.

### 3.4. Influences of $Na_i$ on Bifurcation of SAN Cells

*3.4.1. Enhancing $I_f$ caused increases in $Na_i$*

We further examined the $g_f$-dependent changes in $Na_i$ at EPs and during spontaneous firings in the $Na_i$-variable system by constructing one-parameter BDs in which $Na_i$ at EPs and during spontaneous oscillations were plotted as functions of $g_f$. The values of $Na_i$ at EPs and during spontaneous firings became higher with increasing $g_f$.

*3.4.2. Increasing $Na_i$ shrank parameter regions of unstable EPs and stable LCs*

Because $I_{NCX}$, $I_{NaK}$ and other $Na^+$ fluxes depends on $Na_i$, stability and bifurcations of SAN cells during $g_f$ changes

may be affected by concomitant variations in $Na_i$. We therefore investigated how the parameter $Na_i$ affects stability and bifurcations of EPs and LCs in the $Na_i$-fixed system by constructing two-parameter BDs for $Na_i$ and $g_{CaL}$. The $g_{CaL}$ regions of unstable EPs and rhythmic firings dramatically shrank with increasing $Na_i$.

*3.4.3. $Na_i$-dependent effects of $I_f$ on robustness against hyperpolarizing loads*

While $I_f$ enhances SAN cell robustness in the $Na_i$-fixed system at lower $g_f$, $I_f$-dependent changes in $Na_i$ may eliminate and reverse the enhancing effect of $I_f$ in the $Na_i$-variable system. We examined the influences of $I_f$ on stability of EPs and LCs, as well as their bifurcations, in the $Na_i$-variable and $Na_i$-fixed model cells during hyperpolarizing $I_{bias}$ injections and ACh applications. Bifurcations during hyperpolarizing loads of the model cells were tested for broad ranges of $g_f$ by constructing two-parameter BDs for hyperpolarizing $I_{bias}$ and $g_f$.

In the $Na_i$-variable system, the $I_{bias}$ regions of unstable EPs and stable LCs shrank with increasing $g_f$; spontaneous firings became unstable and arrhythmic via destabilization of LCs, and vanished via stabilization of EPs. In contrast, the unstable EP and stable LC regions of the $Na_i$-fixed system were enlarged by $I_f$ at the relatively small $g_f$; however, greater increases in $g_f$ shrank the $I_{bias}$ region of unstable EPs, whereas that of stable LCs was broadened with increasing $g_f$.

## 4. Discussion

### 4.1. Roles of $I_f$ in SAN Pacemaking

*4.1.1. $I_f$ itself does not destabilize an EP*

$I_f$ is expected to contribute to EP destabilization in SAN cells, like $I_{Ca,L}$ and $I_{Na}$ [1,2]. However, increasing $I_f$ did not enlarge but rather shrank the parameter regions of unstable EPs. Thus, $I_f$ itself does not contribute to EP instability, but facilitates EP stabilization. Excess $I_f$ may counteract the destabilizing effect of $I_{st}$ or $I_{Na}$ on EPs.

*4.1.2. $I_f$ enhances SAN cell robustness to hyperpolarizing loads by preventing bifurcations*

Lower conductance $I_f$ enhanced the central SAN cell robustness against ACh-induced hyperpolarization by preventing emergence of a stable EP. Nevertheless, the $I_f$ effect on the central cell was relatively small. The central cell robustness to hyperpolarizing loads was attenuated at higher $g_f$, suggesting that $I_f$ density should be small in the central region of SAN. In contrast, the peripheral cell showed continuous enlargements of the [ACh] and $G_C$ regions of unstable EPs and rhythmic firings during $I_f$ enhancement. Thus, $I_f$ may enhance the robustness of peripheral SAN cells against hyperpolarizing loads by preventing EP stabilization and LC destabilization.

Previous studies revealed sinus dysrhythmia, recurrent sinus pause, and cessation of spontaneous activity in mice lacking HCN2 or HCN4 [11,12], possibly reflecting $I_f$-induced enhancement of the SAN cell robustness against hyperpolarizing loads. The repetitive sinus pause was prominent at low heart rates, e.g., under muscarinic stimulation or in the presence of $I_f$ blockers [11,12]. These arrhythmic behaviors are very similar to those of the hyperpolarized model cells reproduced in this study.

*4.1.3. Regional differences in $I_f$ effects suggest different roles of $I_f$ in center and periphery*

$I_f$-induced enhancement of the SAN cell robustness was relatively small in the center, but relatively large in the periphery. The greater effect of $I_f$ on the peripheral cell robustness to electrotonic loads and higher $I_f$ density in the periphery are reasonable, because peripheral cells directly suffer the electrotonic load of the atrium and thus must be more robust to electrotonic modulations than central cells. These regional differences in the $I_f$ effects may reflect different roles of $I_f$ in the center and periphery of the SAN: $I_f$ may contribute mainly to the robust pacemaking against electrotonic loads in the periphery, but mainly to the sympathetic regulation of pacemaker frequency in the center.

*4.1.4. $I_{st}$ and $I_{Na}$ are involved in $I_f$–induced enhancement of SAN cell robustness*

$I_f$ was suggested to enhance the central SAN cell robustness against parasympathetic stimulation and the peripheral cell robustness against electrotonic loads of the atrium in combination with $I_{st}$ and $I_{Na}$, respectively. $I_{Na}$-induced destabilization of an EP at hyperpolarizing $V_E$ would be involved in the $I_f$-induced enhancement of the peripheral cell robustness to electrotonic modulations. The combined effects of $I_f$ and $I_{Na}$ may be indispensable for prevention of EP stabilization and robust maintenance of SAN pacemaking against hyperpolarizing loads.

*4.1.5. $I_f$-dependent pacemaking occurs in hyperpolarized cells*

Our results suggest that $I_f$-dependent pacemaking is possible in hyperpolarized cells. Experimental reports suggested the $I_f$-dependent cardiac pacemaker: 1) $Cs^+$, an $I_f$ blocker, abolished spontaneous activity of rabbit SAN cells when hyperpolarizing $I_{bias}$ was applied [13]; 2) the instantaneous background current in the pacemaker potential range was outward before $I_f$ activation in rabbit SAN cells [14]; 3) HCN4-deficient mouse SAN cells were quiescent under low cAMP conditions [11]; and 4) $I_f$-based biological pacemakers could be created in the atrium and ventricle by HCN gene transfer [15]. Thus, bifurcations leading to $I_f$-dependent pacemaking may actually occur in the SAN under hyperpolarized or other non-physiological conditions.

### 4.2. Impacts of $I_f$ on Robustness of SAN Pacemaking

*4.2.1. $I_f$ itself may attenuate robustness of SAN cells*

Larger $I_f$ did not enlarge but rather shrank the [ACh] and hyperpolarizing $I_{bias}$ regions of stable LCs in the $Na_i$-

variable system. This result suggests that $I_f$ does not necessarily enhance the robustness of SAN pacemaking. $I_f$ may contribute mainly to the sympathetic regulation of pacemaker frequency in the center, while contributing to the robust pacemaking against electrotonic loads of the atrium in the periphery.

Overexpression of HCN-encoded pacemaker current was reported to silence biological pacemakers derived from guinea-pig atrial myocytes as a cautionary note for development of $I_f$-based biological pacemakers [16]. This observation may reflect that excess $I_f$ expression yields EP stabilization and thus attenuation of pacemaker cell robustness, as suggested by our study. This finding is of particular importance for $I_f$-based biological pacemaker engineering where $I_f$ could be overexpressed to several times the density of native currents [17].

*4.2.2. $I_f$ effects depend on concomitant changes in $Na_i$*

The differences in the $I_f$ effects between the $Na_i$-variable and $Na_i$-fixed systems come from $I_f$-dependent changes of $Na_i$ in the $Na_i$-variable system; in the $Na_i$-fixed system, the parameter $Na_i$ was shown to exert substantial influences on stability and bifurcations of the model cell via modulating $I_{CaL}$, $I_{NaK}$ and $I_{NCX}$. At lower $g_f$, the decreased $Na_i$ at EPs may contribute to enhancement of SAN cell robustness. On the other hand, the greater $I_f$-dependent shrinkage of the unstable regions in the $Na_i$-variable system is due to the $I_f$-dependent increase in $Na_i$ as observed experimentally [4]. Thus, changes in $Na_i$ strongly affect stability and bifurcations of SAN cells and thus must be taken into account in experimental and theoretical studies.

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