Ionics Mechanisms Underlying the Positive Chronotropy Induced by β1-Adrenergic Stimulation in Guinea Pig Sinoatrial Node Cells: a Simulation Study

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Abstract: Positive chronotropy induced by β1-adrenergic stimulation is achieved by multiple interactions of ion channels and transporters in sinoatrial node pacemaker cells (SANs). To investigate the ionic mechanisms, we updated our SAN model developed in 2003 and incorporated the β1-adrenergic signaling cascade developed by Kuzumoto et al. (2007). Since the slow component of the delayed rectifier K⁺ current (IKs) is one of the major targets of the β1-adrenergic cascade, we developed a guinea pig model with a large IKs. The new model provided a good representation of the experimental characteristics of SANs. A comparison of individual current during diastole recorded before and after β1-adrenergic stimulation clearly showed the negative shift of the L-type Ca²⁺ current (ICaL) takeoff potential, enlargement of the sustained inward current (Ih), and the hyperpolarization-activated nonselective cation current (Ih) played major roles in increasing the firing frequency. Deactivation of IKs during diastole scarcely contributed to the time-dependent decrease in membrane K⁺ conductance, which was the major mechanism for slow diastolic depolarization, as indicated by calculating the instantaneous equilibrium potential (lead potential). This was because the activation of IKs during the preceding action potential was negligibly small. However, the-compromised the positive chronotropic effect by elongating the action potential duration. Enhanced Ca²⁺ release from the sarcoplasmic reticulum failed to induce an obvious chronotropic effect in our model.

Key words: β1-adrenergic receptor, cardiac pacemaker model, sinoatrial node, sympathetic nerve stimulation, simulation.

Sympathetic stimulation of SA node pacemaker cells (SANs) is essential for increasing heart rate when a larger blood supply is required for the body. The autonomic neurotransmitter, noradrenaline, is released from nerve terminals, binds to the β1-adrenergic receptor, and initiates intracellular signal transduction in SANs, which causes the increased firing frequency of spontaneous action potentials. This positive chronotropy is due to a variety of functional modifications of ion channels and ion transporters. To date, electrophysiological and pharmacological studies have provided experimental evidence to show that ion channels and transporters are modified by the β1-adrenergic stimulation. To clarify the contributions of each current, however, an integrative analysis is required because positive chronotropy is induced by multiple interactions of all ion channels and transporters, which have different kinetics and respond differently to β1-adrenergic stimulation. In 2003, we developed a SAN model that included spontaneous action potential generation and intracellular ion homeostasis, including Ca²⁺ dynamics [1, 2]. Using this model, we proposed the principal ionic mechanisms underlying the spontaneous action potential. In the current study, we revised the model by updating the ion channels and incorporating the mitochondria and β1-adrenergic cascade models described by Matsuoka et al. (2004), Terashima et al. (2006), Takeuchi et al. (2006), and Kuzumoto et al. (2007) [3–6] to analyze the roles of each target ion current, including the L-type Ca²⁺ current (ICaL), the sustained inward current (Ih), the hyperpolarization-activated cation current (Ih), and the slow component of the delayed rectifier K⁺ current (IKs) in the positive chronotropy during β1-adrenergic stimulation. Our new model also includes an alteration of Ca²⁺ dynamics during stimulation by calculating a modification of the Ca²⁺ pump (SERCA) on the sarcoplasmic membrane. Thus we discuss the hypothesis that Ca²⁺ release from the sarcoplasmic reticulum (SR) plays a crucial role in regulating pacemaker activity [7–10].

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It is well established that the decay in K⁺ conductance during slow diastolic depolarization is crucial for the generation of spontaneous action potentials [11, 12], and this K⁺ conductance is mainly supported by the rapid component of the delayed rectifier K⁺ current (I_{Kr}) in rabbit. However, Matsuura et al. (2002) [13] demonstrated that the guinea pig SANs show a significant amplitude of I_{Kr} during the depolarizing pulse, and also I_{Kr} tail on repolarization, which was not the case in rabbit SANs (see also Anumonwo et al. (1992) [14]). Furthermore, I_{Kr} is one of the major targets of the β1-adrenergic cascade. Therefore in this study we examined if I_{Kr} in guinea pig could be substituted for I_{Kr} in contributing to pacemaker depolarization during β1-adrenergic chronotropy. We constructed a guinea pig SAN model with the experimental data for I_{Kr} and I_{Ca} obtained from guinea pig SANs by Matsuura et al. [13]. As far as we know, this is the first attempt to investigate the mechanism of the positive chronotropic effect of β1-adrenergic signaling cascades.

**MATERIALS AND METHODS**

**Parameter set in the guinea pig SAN model.** The extracellular ion concentrations (in mM) were 141 for Na⁺, 5.4 for K⁺, 1.8 for Ca²⁺, 140 for Cl⁻, and 10 for large anionic compounds (LA). LA was membrane-impermeable. A schematic diagram of the β1 model is shown in Fig. 1. General abbreviations are listed in the Table S1, the equations for each current. The total background current (I_t) is defined as

\[
I_t = I_{Na} + I_{Ca} + I_{CaT} + I_{at} + I_{ha} + I_{Kl} + I_{Kr} + I_{KS} + I_{KACa} + I_l + I_{NaCa} + I_{NaK} + I_{PmcA} + I_{ICl}
\]

(2)

See Table S10 for definitions and equations for each current. The total background current (I_t) is defined as

\[
I_t = I_{bNSC} + I_{Cab} + I_{Kpf} + I_{I(Ca)} + I_{KATP} + I_{ICl}
\]

(3)

See Table S10 for definitions and equations for each current. The following functions were newly added to the model. First, ATP production by oxidative phosphorylation, creatine kinase, and adenylate kinase [3, 15] (Tables S5 and S15) was implemented. The ATP production rate was adjusted to obtain a steady state [ATP] of 7.0 mM, which was in the range of 5–10 mM [16]. A ratio \([\text{NADH}] / (\text{[NAD]} + \text{[NADH]}) = 0.29\) was within the range of values obtained experimentally in guinea pig ventricular myocytes [17]. Second, the mechanisms of Cl⁻ homeostasis and volume regulation [4, 5] were incorporated by including ion flux via a Na⁺/K⁺/2Cl⁻ cotransporter (J_NKCC1), background Cl⁻ current (I_{Cl}), water flux, and LA. The magnitudes of I_{Cl} and water flux were scaled according to the ratio of input capacitance between SANs and ventricular cells (Tables S4 and S9). The intracellular Cl⁻ concentration was set at about 30 mM by adjusting the activity of J_NKCC1. Since the cystic fibrosis transmembrane conductance regulator (CFTR) Cl⁻ channel current (I_CFTR) was reported to be absent [18] and no data were available for I_CFTR in SANs, they were not included in our model. Equations for the protein kinase A (PKA) and the cAMP effect on I_a and I_{ha} were newly implemented in the

**Fig. 1. Schematic diagram of the guinea pig SA node cell model.**
SAN model. And third, the β1-adrenergic signaling module [6] (Tables S6 and S12) was incorporated in the model. Target proteins of β1-adrenergic signaling, including $\text{IK}_s$, are colored in red in the diagram (Fig. 1). One significant feature of guinea pig SANs is a larger $\text{IK}_s$, compared to $\text{IK}_{\text{r}}$ as indicated by Anumonwo et al. (1991) [14] and Matsuura et al. (2002) [13], in contrast to rabbit SANs, which has an almost negligible $\text{IK}_s$ [19]. In regard to the kinetic equations for $\text{IK}_r$, we adopted those developed for $\text{IK}_r$ in rabbit by Ono and Ito (1995) [20].

Ordinary differential equations were integrated using the Euler method with an adaptive time step, and simultaneous equations were solved by Broyden’s method, a quasi-Newton procedure. The model was constructed with a Java-based simulation platform, simBio [21]. The source code is available at http://www.sim-bio.org/.

Ionic channels and transporters modulated in the β1-adrenergic signaling cascade model. In our SAN model, $I_{\text{Ca}L}$, $I_{\text{Na}K}$, $I_{\text{K}r}$, $I_{\text{Na}r}$, the plasma membrane Ca$^{2+}$-ATPase (PMCA) current ($I_{\text{PMCA}}$) and the SR Ca$^{2+}$ pump (SERCA) current ($I_{\text{SR}A}$) are enhanced by β1-adrenergic stimulation. Such stimulation activates adenylate cyclase via G-protein (Gs) and elevates the cAMP level. The increased cAMP activates PKA by unleashing its catalytic subunits to phosphorylate the above target proteins, except for $I_{\text{KCa}}$, which is directly modulated by the concentration of cAMP. In the simulation, the activities of $I_{\text{Ca}L}$, $I_{\text{Na}K}$, $I_{\text{K}r}$, $I_{\text{Na}r}$, $I_{\text{PMCA}}$, and SERCA ($I_{\text{SR}A}$) were modulated as a function of the concentration of PKA catalytic subunits or cAMP, as follows.

(1) $I_{\text{Ca}L}$: Equations for $I_{\text{Ca}L}$ were basically the same as those used in the ventricular cell model [6]. The increase of $I_{\text{Ca}L}$ and negative shift of current-voltage (I-V) relationships by β1-adrenoceptor stimulation were reconstructed by defining model equations as functions of [PKA], the concentration of free catalytic subunit of PKA. The fully activated amplitude of $I_{\text{Ca}L}$ was multiplied by $f_{\text{PKA}}$, which is given as (Table S7),

$$f_{\text{PKA}} = \max \cdot \frac{[\text{PKA}]-\text{PKA}_0}{[\text{PKA}]-\text{PKA}_0}^n + 1$$

where the value for the concentration of PKA in the absence of β1-adrenergic stimulation (PKA = 0.000136 mM) was obtained from the original paper by Kameyama et al. (1985) [22] and other values for the maximum amplitude (max), Hill coefficient (n) and the equilibrium constant for PKA (KmPKA) were determined to reconstruct the recorded increase of $I_{\text{Ca}L}$ by ISO described by Zaza et al. (1996) [23] and Ke et al. (2007) [24] in SANs (17.5, 1 and 0.0065 mM, respectively).

The voltage shift of gating was given by biasing $V_m$ with the following $V_{\text{shift}}$ in all equations defining the rate constants of the voltage-dependent gate (Table S7):

$$V_{\text{shift}} = 62.5 \cdot \frac{([\text{PKA}]-\text{PKA}_0)^n}{([\text{PKA}]-\text{PKA}_0)^n + K_m^{\text{PKA}^n} + 1}$$

Parameters for Ca$^{2+}$-dependent inactivation gate were adjusted to obtain the experimental inactivation time course of $I_{\text{Ca}L}$, as shown in Fig. 2A [24]. In both simulation and
experiment, the amplitudes were approximately doubled by adding 0.1 μM isoprenaline (ISO). The negative shift of the peak I-V relationship was approximately 7 mV (Fig. 2B, upper panel), as obtained experimentally in guinea-pig SANs (Fig. 2B, lower panel) [24] and in various types of cells [25]. In the dose-response curve in Fig. 2C, saturated increase of \( I_{CaL} \) was 116% of the control.

(2) \( I_{st} \): The increase of \( I_{st} \) was reconstructed by defining the limiting amplitude as a function of [PKA] (Table S7). The fully activated amplitude of \( I_{st} \) was multiplied by \( f_{PKA} \), which is given by the same equation for \( I_{CaL} \) (Eq. 4), but values for max and \( K_{m} \)PKA were different from those of \( I_{CaL} \) (1.0 and 0.000065 mM, respectively). In Fig. 3, currents in the presence of 0.1 μM ISO (●) were approximately doubled compared to the control (○) in both the simulation and the experiment. The I-V relationships in Fig. 3B were reconstructed with and without ISO by plotting the amplitude of the initial peak \( I_{st} \) measured at the beginning of depolarizing pulses. The reversal potential of \( I_{st} \) was +12 mV. The amplitudes of the current were increased in a dose-dependent manner as shown in Fig. 3C, in agreement with the experimental data of Toyoda et al. (2006) [26].

(3) \( I_{ha} \): The positive shift in voltage-dependent activation of \( I_{ha} \) was defined as a function of the concentration of free cAMP, [cAMP] (Table S7). As for \( I_{CaL} \), the voltage

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**Fig. 3.** Comparison of \( I_{st} \). (A) Voltage clamp records of \( I_{st} \) with (●) and without (○) 0.1 μM ISO in simulations (upper panel) and experiments [30] (lower panel). (B) Simulated peak I-V relationships of \( I_{st} \) with (red line) and without (black line) 0.1 μM ISO. The holding potential was –80 mV. (C) ISO concentration–\( I_{st} \) relationship compared to experimental data (mean ± SEM) [26].

**Fig. 4.** Comparison of \( I_{ha} \). (A) Voltage clamp records of \( I_{ha} \) with (●) and without (○) ISO in simulations (upper panel) and experiments [27] (lower panel). ISO concentrations were 0.1 μM in the simulation and 1 μM experimentally. Traces were elicited by 2 s test pulses to –80 mV from a holding potential of –35 mV. (B) Simulated activation-voltage relationships of \( I_{ha} \) with (red line) and without (black line) 1 μM ISO are compared with experimental I-V relationships in the lower panel (with [●] and without [○] 1 μM ISO) [28]. Data were obtained at the end of 4 s hyperpolarizing pulses to various test potentials from a holding potential of –40 mV.
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The shift of gating was given by biasing $V_m$ with $V_{shift}$ (calculated as follows) in all equations defining the rate constants of the voltage dependent gate,

$$V_{shift} = 15 \cdot ( [cAMP] - cAMP_0 )^{nH} / ( [cAMP] - cAMP_0 )^{nH} + K_{mcAMP}^{nH}$$

where values for the concentration of cAMP in the absence of β1-adrenergic stimulation (cAMP0), nH and the equilibrium constant for cAMP (K_{mcAMP}) were 0.000294 mM, 1 and 0.0002 mM, respectively. [cAMP] represents the concentration of free cAMP. In Fig. 4A, $I_{ha}$ activated at –80 mV in the presence of 0.1 µM ISO ([cAMP] = 4.88 µM) was larger than the control, as in experiments [27] shown in the lower panel. The simulated activation time course of $I_{ha}$ in the presence of ISO was accelerated slightly. The voltage-shift of activation in the simulation was similar to that in the experimental data, as shown in Fig. 4B [28]. The $V_{1/2}$ of the sigmoid curves obtained in the control and in the presence of 1 µM ISO were –69.1 mV and –43.7 mV, respectively, in the simulation (upper panel) and –61.9 mV and –47.1 mV, respectively, in experiments (lower panel). It should be noted that $I_{ha}$ could be activated over the voltage range of slow diastolic depolarization in the presence of β1-adrenergic stimulation.

$\beta_1$-Adrenergic Stimulation: An $I_{Ks}$ model of guinea-pig SANs is a modification of the recently developed new ventricular model [6] (Table S8). Channel gating was described by voltage-dependent and Ca2+-dependent activation kinetics, and β1-adrenergic stimulation modifies the voltage gate and the current amplitude, as in the ventricular cell model. The voltage dependency and the permeability of the ventricular $I_{Ks}$ model was modified according to experimental data obtained by Matsuura et al. (2002) [13]. Figure 5A compares the time courses of $I_{Ks}$ activation and deactivation induced by the pulse protocol indicated at the top of the figure. The very slow component of activation was not observed in simulation, but the initial activation time course was similar to those in the experiment. The deactivation time course over the diastolic potential range also agreed with experimental data; it was fitted well by a sum of two exponential functions with time constants of $\tau_a = 61.0$ ms and $\tau_s = 122.7$ ms at –50 mV, compared to 44.4 and 151.5 ms in the experiment. (B) I-V relationships for $I_{Ks}$ tail currents obtained with (red line) and without (black line) application of 1.0 µM ISO are compared with experimental I-V relationships [29] in the lower panel (with [●] and without [□] 1 µM ISO). Data points were obtained at the end of 4 s depolarizing pulses to various test potentials from a holding potential of –50 mV.
pendency in a negative direction. The fully activated amplitude of $I_{Ks}$ was multiplied by $f_{PKA}$, which is given as a function of a fractional concentration of phosphorylated KCNQ1, $F_{\text{KCNQ1p}}$,

$$F_{\text{KCNQ1p}} = \frac{[\text{KCNQ1p}]}{[\text{KCNQ1}_{\text{tot}}]},$$

(7)

$$f_{PKA} = 0.565 / \{1 + (0.4 / F_{\text{KCNQ1p}})^6 \} + 0.435.$$  

(8)

Similarly, the voltage shift was defined by $F_{\text{KCNQ1p}}$ as,

$$V_{\text{shift}} = 1.35 \cdot \{ (F_{\text{KCNQ1p}} / 0.0947) – 1 \}.\quad (9)$$

In Fig. 5B, $I_{Ks}$ tail current increased 1.5- to 2-fold by 1 µM ISO in the voltage range of action potential, which agreed with experimental data [29].

(5) $I_{NaK}$, $I_{SR}$, and $I_{PMCA}$: Because of insufficient data for $I_{NaK}$, $I_{SR}$, and $I_{PMCA}$ in guinea-pig SANs, we used essentially the same equations for kinetics as those in the ventricular cell model [6] (Table S11).

RESULTS

Whole cell current and current-voltage relationship in the SAN model

The simulated membrane currents and current-voltage relationships obtained from the guinea pig SAN model (upper panel) are compared with experimental results (lower panel) from Guo et al. (1997) [30] in Fig. 6. Hyperpolarizing pulses from the holding potential of –50 mV activated the slowly increasing inward current, $I_{Ks}$, which became visible from –70 to –80 mV. The activation time courses seemed similar, except for faster activation in the experiment at –110 mV. Depolarizing pulses to various potentials induced transient inward current, which was attributed to $I_{CaL}$, and slowly increased the outward current, $I_{Ks}$. The activation threshold of $I_{CaL}$ was –40 mV, and the potential of the peak inward current in the I-V curve was –10 mV in both the simulation and experiment (*) . The voltage range of delayed rectification in the simulation also agreed well with the experimental I-V relationship for the late current (○). On repolarization, outward tail currents were observed as a result of the deactivation of $I_{Ks}$ and $I_{Kf}$. The contribution of $I_{Kf}$ to the tail current was comparable to that of $I_{Ks}$; the ratio of $I_{Kf}/I_{Ks}$ was 0.74 after the depolarizing pulse to +20 mV. This is in contrast to the major contribution of $I_{Ks}$ to the onset current during the depolarizing pulse; $I_{Kf}/I_{Ks}$ was 0.02 during the depolarizing pulse to +20 mV because of the strong inward rectification of $I_{Kf}$.

Ionic mechanisms of spontaneous action potentials

The spontaneous activity of the cell was successfully reproduced as a result of the coordination of all the ion channels and transporters in the model. The action potential parameters are compared in Table 1. Both the maximum diastolic potential and the overshoot are in good agreement with those in the experiments. The maximum rate of rise in the simulation is within the range of experimental data reported by Guo et al. (1997) [30] and Toyoda et al. (2005) [26], but slightly larger than that of Rigg et al. (2000) [31], and the spontaneous frequency agrees well with Rigg et al. (2000) [31]. To understand the mechanism underlying pacemaker depolarization, we adopted the “lead potential, $V_L$” analysis introduced by Sarai et al. (2003) [2]. The lead potential is defined by the equivalent circuit shown in Fig. 7A, where $dV_m/dt$ is given as

$$dV_m \over dt = -G_m \cdot (V_m - V_L) \over C_m.$$

(10)

Both $V_L$ and $G_m$ vary automatically in the SAN model ac-
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According to the time-dependent changes in membrane ionic conductance ($G_m$) and the equilibrium potential ($E_X$) for each ionic species (X):

$$G_m = G_K + G_{Na} + G_{Ca} + G_{Cl},$$

$$V_L = \frac{G_K E_K + G_{Na} E_{Na} + G_{Ca} E_{Ca} + G_{Cl} E_{Cl} - INaK - IPMCA}{G_m},$$

When $G_K$, $G_{Na}$, $G_{Ca}$, and $G_{Cl}$ were calculated from the total K⁺, Na⁺, Ca²⁺, and Cl⁻ fluxes (Table S4), respectively, along the time course of the spontaneous action potential, $V_L$ changed as shown in the control panel in Fig. 7B ($V_L$ in red and $V_m$ in gray). When $dV_m/dt$ is positive, $V_L$ is more positive than $V_m$, so that an inward current is generated and the membrane depolarizes, with the opposite effect of when $dV_m/dt$ is negative. $V_L$ crossed $V_m$ at all flexion points where $dV_m/dt$ is zero at the overshoot potential and the maximum diastolic potential.

To evaluate the contribution of individual currents to the slow diastolic depolarization by $V_L$ analysis, the gating parameter for a given current was fixed for 275 ms (unshaded).

![Diagram](Image)

**Table 1.** Action potential parameters of single guinea pig SANs. In the simulation, parameters were obtained in the steady state for control data and 30 s after the application of 0.01 µM and 0.1 µM ISO.

| Reference            | Maximum diastolic potential (mV) | Overshoot (mV) | Total amplitude (mV) | Maximum rate of rise (V/s) | Frequency (/min) |
|----------------------|----------------------------------|----------------|----------------------|----------------------------|-----------------|
| **Control**          |                                  |                |                      |                            |                 |
| Rigg et al. (2000) [31] | –57 ± 2                        | 16 ± 4         | 73                   | 1.5 ± 0.4                  | 132 ± 19        |
| [ISO] 0.1 µM         | –52 ± 2                         | 27 ± 4         | 89                   | 3.5 ± 0.6                  | 284 ± 14        |
| Guo et al. (1997) [30] | –58.7 ± 3.8                    | 17.9 ± 4.8     | 76.6                 | 3.6 ± 1.3                  | 191 ± 76.6      |
| Toyoda et al. (2005) [26] | –64.3*                        | 17.5*          | 81.8                 | 7.0 ± 1.2                  | 178 ± 8         |
| Matsuura et al. (2002) [13] | –64.3 ± 1.8*                  | 22.5*          | 86.8                 | –                         | 205 ± 11        |
| **ISO**              |                                  |                |                      |                            |                 |
| [ISO] 0.01 µM        | –65.5                           | 13.69          | 79.19                | 5.117                      | 159.9           |
| [ISO] 0.1 µM         | –66.12                          | 15.04          | 81.16                | 7.29                       | 177.6           |

*Data were corrected for a liquid junction potential of 5 mV between the aspartate-rich pipette solution and the Tyrode solution. The results were expressed as the mean ± SEM except for results obtained by Guo et al., which were expressed as the mean ± SD.
shaded period) at the value obtained at the maximum diastolic potential. Note that the calculation of \( Vm \) (blue line) was independent from that of \( Vm \) (gray line); thus the time course of \( Vm \) is the same in all panels. When the gating parameter of \( IKr \) was fixed, \( Vm \) became flat below \( Vm \), indicating that \( IKr \) is mainly responsible for slow diastolic depolarization. In contrast, when the gating parameter of \( IKs \) was fixed, the time course of \( Vm \) was only moderately modified if compared with the control \( Vm \). \( ICaL \) played an important role in accelerating the last phase of slow diastolic depolarization, which was in agreement with its activation threshold at around –40 mV. \( ICaL \) played a crucial role in determining the depolarizing rate in the latter half of slow diastolic depolarization (Fig. 8). These findings are in good agreement with a previous study in the “rabbit” SAN model [2].

To gain a deeper insight into the contribution of \( IKr \) to the rate of slow diastolic depolarization in comparison to \( IKs \), open probabilities and amplitudes of \( IKr \) and \( IKs \) were compared during slow diastolic depolarization (Fig. 8). The driving force for outward K+ flux increased in a time-dependent manner, but the open probability of \( IKr \) decreased gradually from 0.46 to 0.10 (Fig. 8B); thus the current amplitudes of \( IKr \) remained fairly constant in the late phase of pacemaker depolarization (Fig. 8C). As expected from the deactivation time course of \( IKr \) in the voltage clamp experiment (Fig. 5), the open probability of \( IKr \) also decreased during diastolic depolarization (Fig. 8D). However, the amplitude of \( IKr \) was very small after the time of the maximum diastolic potential. This was because the open probability of \( IKr \) during the action potential was only 0.007 at the maximum, which was 1/58.8 of \( IKr \) because of its slow activation kinetics. Furthermore, the reversal potential of \( IKr \) was less negative than that of \( IKs \), giving a smaller electrochemical driving force for \( IKs \) during diastole. Thus \( IKr \) could not contribute to the decay in the whole cell K+ conductance during slow diastolic depolarization (Fig. 8E).

### Time course of responses to β1-adrenergic stimulation

The time course of the β1-adrenergic effect is one of the critical factors in controlling arterial blood pressure or in evaluating autonomic regulation using the R-R interval variability. Here the time course of firing frequency of SANs was examined in terms of underlying reaction cascade in the model (Fig. 9).

The concentration of cAMP increased exponentially within the initial 20 s after the onset of ISO application, as shown in the top panel of Fig. 9A, and its saturation level was determined by the phosphodiesterase (PDE) activity. The fraction of the free catalytic subunit of PKA showed almost the same time course as that of cAMP (Fig. 9A, middle panel). Approximately 13% of the activated catalytic subunit was inactivated by the binding of protein kinase inhibitor (PKI) at 30 s after the application of 0.01 µM ISO. The increase of firing frequency saturated much earlier than those of cAMP or PKA activation after ISO application (Fig. 9A, bottom panel). This dissociation of the time course in firing frequency from that in cAMP or PKA is due to the nonlinear relationship between the channel activation and the firing frequency; however, the details of the mechanism are currently unclear. We were only able to test the secondary effect of a modified intracellular Na+ concentration ([Na+]i), which increased almost linearly to 5.13 mM, from 4.45, within the initial 30 s of ISO application. Indeed, the outward \( INaK \) modulated by activated PKA, was gradually enlarged by the increase in [Na+]i (Fig. 9B, top panel). When the amplitude of \( INaK \) was temporarily decreased to the initial level (Fig. 9B, middle panel) by arbitrarily decreasing its magnitude factor, the firing frequency was increased. The enhancement of \( INaK \) by increasing the concentration of intracellular Na+ was one of the mechanisms responsible for the early saturation in the acceleration of firing frequency.

The relationship between the increase in firing frequency and ISO concentration is shown in Fig. 9C. The firing
The frequency was measured 30 s after the application of ISO, which increased the frequency by 41.5% from the control rate at the maximum dose (10 µM) of ISO. Experimental data from Rigg et al. (2000) [31] were superimposed on the simulation result. The curve obtained from simulation \( n_H = 0.586, K_D = 0.0098 \) µM almost coincided with the experimental result in the presence of 2 µM ryanodine (○) in the low concentration range below 0.01 µM, but the slope was shallower than that obtained without ryanodine (●). The approximate half-maximal dose for ISO, 0.01 µM, was used in further analyses in this study.

The parameters of action potentials in simulations in ISO (0.01 and 0.1 µM) are compared in the Table 1 with experimental data (0.1 µM) in isolated guinea pig SANs from Rigg et al. [31]. The maximum diastolic potential, overshoot, total amplitude, maximum rate of rise, and frequency increased in both the simulation and the experiment in the presence of ISO. The increase in firing frequency in the simulation was smaller than that in the experiment.

In the current model, \( J_{NKCC1} \) and \( I_{CIB} \) are not modified by β1-adrenergic stimulation. However, \([CIT]_i\) increased due to changes in \( I_{CIB} \) which is dependent on \( V_m \), from a control level of 30.04 mM to 30.06 mM after the application of ISO for 2 h. Because the change in \([CIT]_i\) was small, we concluded that the change in the cell volume was negligibly small.

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\( V_m \) and currents obtained in control (black line) and 30 s after 0.01 µM ISO application (red line) are superimposed individually at the time point of the maximum rate of rise in Fig. 10. The slope of slow diastolic depolarization was significantly increased by ISO application to 0.126 mV/ms, from 0.087, when measured over the linear portion of the diastolic depolarization. This increase in the depolarizing rate (0.039 mV/ms) can decrease the duration taken for ~25 mV depolarization from the maximum diastolic potential (~65 mV) to the takeoff potential of the action potential.
The negative shift of \( I_{\text{CaL}} \) activation (\( \nu_{\text{shift}} = 1.7 \text{ mV with } 0.378 \mu\text{M catalytic subunit of PKA} \)) is marginal (1.7/25 = 6.8%) if compared with the 25 mV magnitude of the diastolic depolarization. Thus we conclude that the increase in the slope of diastolic depolarization is mainly responsible for the positive chronotropy.

A prolongation of the action potential by about 15.4 ms partially compromised the positive chronotropic effect of the increased rate of diastolic depolarization.

The increase in the rate of diastolic depolarization is due to increases in both \( I_{\text{st}} \) and \( I_{\text{ha}} \). If measured over the period from the maximum diastolic potential to the take-off potential, the ratio of the increase of \( I_{\text{st}} / I_{\text{ha}} \) measured by the time integral of the increase of each current during slow diastolic depolarization was 1.19, indicating almost equal contributions of these currents to the positive chronotropy.

The increase in the outward \( I_{\text{Kr}} \) during diastole is secondarily caused by the positive shift of \( V_m \). On the other hand, the amplitude of \( I_{\text{ks}} \) increased by up to 150% at the peak directly by the PKA catalytic subunit and activation of the Ca\(^{2+}\)-dependent gate. The current amplitude of \( I_{\text{ks}} \) was still negligibly small during diastolic depolarization after \( \beta_1 \)-adrenergic stimulation.

Intracellular Ca\(^{2+}\) concentration, \( I_{\text{NaCa}} \), and \( V_m \) are shown in the top, middle, and bottom panels, respectively. The traces were recorded for control conditions (black lines) and 30 s after the application of 0.01 \( \mu\text{M ISO} \) (red lines).

The increase in \( I_{\text{NaCa}} \) by \( \beta_1 \)-adrenergic stimulation increased the Ca\(^{2+}\) content of the cell, especially within the SR, which then increased the magnitude of the Ca\(^{2+}\) transient by 56% at the peak, thereby increas-


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The magnitude of $I_{NaCa}$ during the Ca$^{2+}$ transient. However, because of the PKA-dependent activation of Ca$^{2+}$ uptake by SERCA, the decay of the Ca$^{2+}$ transient accelerated during β1-adrenergic stimulation, so that [Ca$^{2+}$]$_i$ and therefore the magnitude of inward $I_{NaCa}$ were almost superimposable with control data during diastolic depolarization (Fig. 11, middle panel). Thus the above modulation of $I_{NaCa}$ does not contribute to the positive chronotropy in the model. On the contrary, the increase in $I_{NaCa}$ during the action potential hampered the positive chronotropic effect of β1-adrenergic stimulation by prolonging the action potential duration. These conclusions differ from those in Kurata et al. (2002) [32] and Maltsev et al. (2004) [33] and will be discussed in the next section.

**DISCUSSION**

$I_{Ks}$ and $I_{Kr}$ in the guinea pig SAN cell model

$I_{Ks}$ has recently been suggested to be a large component of $I_K$ in guinea pig SANs [13, 14], and therefore we investigated the contribution of $I_{Ks}$ to slow diastolic depolarization by simulation. This analysis showed that the decay of K$^+$ conductance by the deactivation of $I_{Kr}$, but not by $I_{Ks}$, only influenced the lead potential, $V_L$, and played a major role in determining pacemaker depolarization. These conclusions differ from those in Kurata et al. (2002) [32] and Maltsev et al. (2004) [33] and will be discussed in the next section.

Intracellular Ca$^{2+}$ concentration and pacemaker activity

It is still a matter of debate whether intracellular Ca$^{2+}$ dynamics affect pacemaker activity [9, 10, 36]. The mechanism in which the inward $I_{NaCa}$ was enhanced by the time-dependent local intracellular Ca$^{2+}$ concentration and accelerated pacemaker depolarization has been reproduced only in the model developed by Kurata et al. (2002) [32]. In this model, it was assumed that the subsarcolemmal domain of 1% of the cell volume increased the Ca$^{2+}$ concentration-dependent enhancement of $I_{NaCa}$ during the last 60 ms of slow diastolic depolarization, and that activated $I_{CaT}$ increased the local subsarcolemmal Ca$^{2+}$ concentration in addition to SR Ca$^{2+}$ release during slow diastolic depolarization. However, without the effect of the subsarcolemmal domain (as in our model), Ca$^{2+}$ released from the SR diffuses to the cytosol instantaneously and does not enhance $I_{NaCa}$. Similarly, the inclusion of diastolic spontaneous local Ca$^{2+}$ release from the SR, as measured by Bogdanov et al. (2001) [37], in a model includ-
ing the subsarcolemmal space increases the rate of slow diastolic depolarization by activating $I_{\text{NaCa}}$ [33]. To assess the validity of different models, we need to determine experimentally if the subsarcolemmal Ca$^{2+}$ concentration is changed when EGTA is applied to inhibit the global (myoplasmic) Ca$^{2+}$ transient. This is because most of the spontaneous action potentials have been recorded using the ruptured patch clamp mode with EGTA in the pipette solution. The amplitude of $I_{\text{CaL}}$ is still uncertain in guinea pig SANs, and further experiments are needed. It is also important to consider variability in the distributions of ionic channels [36] according to the location and size of the cells [38] when the contribution of the SR to pacemaker activity is being discussed [39].

### Spontaneous activity and mechanisms of positive chronotropy during β1-adrenergic stimulation in SANs

The positive chronotropic effect of β1-adrenergic stimulation is summarized in Fig. 12, using the scheme of sequential events during the spontaneous action potential. The negative shift of $I_{\text{CaL}}$ activation, which supports the initial rising phase and the overshoot of the action potential [40], was only supplemental to the β1-adrenergic chronotropy at moderate agonist concentrations. The increase in the $I_{\text{CaL}}$ magnitude prolongs the duration of action potentials and thereby decreases the firing frequency, as suggested by Sarai et al. (2003) [2]. In turn, the increased intracellular Ca$^{2+}$ transient under β1-adrenergic stimulation partly compensated for the increase in the action potential duration by inactivating $I_{\text{CaL}}$ and activating $I_{\text{Ks}}$. The increase in $I_{\text{NaCa}}$ and $I_{\text{p}}$ contributed equally to accelerating slow diastolic depolarization. The contribution of $I_{\text{NaCa}}$ to modifying slow diastolic depolarization was almost negligible in our model, even in the presence of β1-adrenergic agonist. However, such currents might play a crucial role in increasing the rate of depolarization only if $I_{\text{NaCa}}$ is strongly coupled with SR Ca$^{2+}$ release by assuming a very limited subsarcolemmal space, as in the model of Kurata et al. (2002) [32] and in the modified version of this model in Maltsev et al. (2004) [33].

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